AGRIVITA
Journal of Agricultural Science
www.agrivita.ub.ac.id

Morphological Characterization and *Fusarium* Wilt Resistance of Triploid Banana Mutant Line (*Musa acuminata* Cola)

Ishak Ishak[1*)] and Jumjunidang[2)]

[1)] Research Organization for Nuclear Energy, National Research and Innovation Agency, Indonesia
[2)] Research Organization for Horticultural and Food crops, National Research and Innovation Agency, Indonesia

ARTICLE INFO

Keywords:
Br 23 banana mutant line
Fusarium wilt resistance
Morphological characterization

Article History:
Received: August 18, 2022
Accepted: December 15, 2023

*) Corresponding author:
E-mail: ishakazra@yahoo.com

ABSTRACT

Br 23 banana mutant line derived from irradiated gamma-ray from pisang ambon cultivar was triploid (AAA) and sterile. Therefore, application of conventional breeding to improve the agronomic characteristics of bananas requires strenuous effort. Morphological and agronomical characterizations of mutant line were observed during the generative stage on the Fusarium hotspot. Sixteen qualitative characters were observed, and each of which related to color was standardized using RHS color chart. Based on these qualitative characteristics, it was confirmed that pisang Ambon belongs to the acuminata group. Several agronomical characteristics of Br 23 banana mutant line, such as the number of hands/bunch, the weight of fruits/hand, and single fruit weight, were observed. The experimental results on banana production showed that the first harvest could reach 26 t/ha. Unlike from the control plant, the Br 23 mutant line showed tolerant characteristics to Fusarium TR4. The evaluation of susceptibility to Fusarium wilt was analyzed using the Vegetative Compatibility Group (VCG) method with isolate no. 01236/16. These susceptible plants were planted nearby the Br 23 banana mutant line. In early 2019, this Br 23 banana mutant line was registered as a new mutant variety under the name of PIRAMA I.

INTRODUCTION

Popularly known as pisang in Bahasa Indonesia, numerous banana's cultivars can be cooked and edible. Most edible bananas, such as pisang ambon, belong to the acuminata group with the AAA genome. There are pisang ambon landraces with different morphological characteristics, such as pisang ambon lumut cultivar, which have green peel even when the fruit is ripe. While, pisang ambon kuning cultivar has yellowish peel color when the fruit is ripe. However, no report is available on the detail morphological characterizations of these pisang ambon cultivars. Therefore, this paper aimed to supply a detail morphological description of pisang ambon.

Bananas, such as pisang ambon and barangan cultivars have an economic potential as a commodity for further development. According to the data released by the Indonesian Ministry of Agriculture in 2016, the harvested area of bananas in Indonesia almost reached 157 thousand ha in 1980, and in 2015, it decreased to only 94 thousand ha, or nearly decreased at 63 thousand ha. Furthermore, it increased in 2017, reaching up to 59.36% compared to 1980 (Rohmah, 2016). The development of the banana commodity is promising as long as there are technological stimuli to improve the fruit quality and its competitiveness at the world market.

Pisang ambon and barangan cultivars have relatively high economic potential value. Compared to pisang ambon, pisang barangan cultivar can be stored longer because of its relatively thick peel, which is beneficial for consumers and for export purposes. Unfortunately, both pisang ambon and

ISSN: 0126-0537

Cite this as: Ishak, I., & Jumjunidang. (2024). Morphological characterization and *Fusarium* wilt resistance of triploid banana mutant line (*Musa acuminata* Cola). *AGRIVITA Journal of Agricultural Science, 46*(1), 121-133. http://doi.org/10.17503/agrivita.v46i1.3900

barangan cultivars are susceptible to Fusarium wilt disease, especially Fusarium race 4. Fusarium wilt was first identified in 1933 in Jamaica. The fungus attacked the "gros michel" banana type as reported by Ploetz (2015). Gros michel type, which has a similar ploidy level as pisang ambon with AAA (triploid) and parthenocarpy, is susceptible to Fusarium wilt (Wang et al., 2012), transcriptional changes occurred in response to *Fusarium oxysporum* f. sp. cubense (Foc) attacks. The basic defense mechanism involved high-level transcription in recognizing fungus attacks (Li et al., 2012). Whereas Dita et al. (2018) mentioned that a new variance Foc called tropical race 4 (TR4) attacked the cavendish banana. This fungus grows and develops in East and Southeast Asia regions. TR4 also infects other cultivars with the AAA genome.

Fusarium wilt disease, which is caused by *Fusarium oxysporum* f.sp *cubense* (Foc), a soil-borne fungus, is harmful banana because it can devastate banana's plantation in relatively short time. Several banana-producing countries, such as Panama, Indonesia, Costa Rica, and Brazil are threatened by the disease. In the mid-twentieth century, *Fusarium oxysporum* f. sp. cubense (Foc) race 1, also known as Panama disease, wiped out the gros michel banana industry in Central America (Dita et al., 2018). The devastation caused by Foc race 1 was mitigated by a shift to resistant cavendish cultivars, which are currently the source of 99% of banana exports (Dita et al., 2018). Fusarium wilt has been threatening the banana industry for many years, with devastating effects on the economy of many tropical countries, and becoming the leading cause of changes in land use in severely affected areas (Olivares et al., 2021).

Fusarium oxysporum f. sp *cubence* (Foc) consists of four races. Race 1 attacks the AAA group banana plant, while race 2 attacks the AAB banana plant like pisang kepok. Meanwhile, race 3 attacks the ornamental banana plant (Heliconia), and race 4 attacks pisang ambon and cavendish (Pegg et al., 2019). Subtropical race 4 (STR4) also affects these cultivars as well as cavendish cultivars (AAA). A lot of bananas in Indonesia have an AAA genome, such as pisang ambon, barangan, and pisang bamban cultivar from West Sumatra. (Maryani et al., 2019) mentioned that more than 65 % of the isolates diagnosed as tropical race 4 (Foc-TR4) were Indonesian origin.

The main problem of growing bananas from the acuminata group is their sensitivity to Fusarium wilt disease. Therefore, efforts should be made to improve their genetical tolerance. As previously mentioned, it is difficult to breed bananas conventionally because these plants are generally parthenocarpy and triploids, which do not have seeds. One of the alternatives for genetic improvement of bananas against Fusarium wilt disease is through induced mutations technique or genetic engineering. The induced mutation of banana has been carried out by deploying embryogenic cell suspension culture (ECS) for plant regeneration as well as a mutant selection during the acclimatization phase and under field conditions (López et al., 2017). The enhanced plant regeneration through *in vitro* culture can be obtained through plant hormone modification (Yusnita, et al., 2015). Besides, mutation breeding to obtain mosaic virus banana-tolerant mutants was reported by El-Sayed et al. (2011). Induced mutations in vegetatively propagated plants are very efficient to obtain genetic diversity. Many horticultural crops, such as chrysanthemum *(Chrysanthemum* spp.*)*, rose *(Rosa* spp.*)*, and carnation *(Dianthus caryophyllus* (Yamaguchi, 2018), and mutant of gladiolus varieties (Sisodia & Singh, 2015), are vegetatively propagated, making it relatively easy to propagate mutants.

This research aims to improve banana traits through the mutation process using gamma rays (Co-60) since nucleotide variation caused by a mutation within the coding frame of the genes can change agronomic traits.

MATERIALS AND METHODS

Plant Propagation

Br 23 banana mutant line clone was propagated at the plant tissue culture laboratory, the Center for Isotopes and Radiation Applications, Jakarta, on 2015-2016. Plant propagation was conducted using Murashige and Skoog's media containing micronutrients, macronutrients, and vitamins, according to Murashige & Skoog (1962), supplemented with BAP (5 mg/l) and IAA (0.5 mg/l). Sterilized shoot-tips were grown on these MS media for 4 to 8 weeks. Some new shoots appearing on the peripheral part of explants were transferred to new fresh media containing BAP (3 mg/l) and IAA (0.5 mg/l) for further proliferation. Subcultures of plantlets were carried out until four generations. Plantlets were then transferred to rooting MS medium containing IBA (3 mg/l) for four weeks.

Plant Acclimatization in Greenhouse

Plantlets with sufficient roots were acclimatized in the greenhouse for 2-3 weeks. Plantlets' roots were washed with tap water and then grown on compost covered with transparent plastic. After the plants were well adapted to the compost medium during a 2-3 week-period, they were then transferred to polybags containing mixture of soil and compost with a ratio of 1:1 (v/v). These plants were then grown for three months.

***Fusarium* Tolerance Evaluation**

Br 23 banana mutant line was previously grown on a hot spot *Fusarium* area that was more than ten years, and the shoot-tip of the suckers was taken for propagation through plant tissue culture techniques. Planting the Br 23 banana clones at the same location in the hot spot area was carried out for the re-testing of the clone's *Fusarium*-resistance. This area is located in South Jakarta, having an altitude of 44 meters above sea level and rainfall ranging from 2000-3000 mm/year. A total of 30 three months-plants planted in polybags were ready to be transferred into the field experiment. Previously, planting holes were prepared at the size of 50 x 50 x 50 cm. The excavated soil was prepared and mixed with 2:1 compost, after previously being mixed with 20 g of NPK fertilizer/hole. The planting interval between plants was set to 2.2 x 2.0 meters. Watering was done every day until the plants were well-adapted to the field. Five susceptible mutants were planted in the same location. *Fusarium* tolerance and sensitive clones were observed during vegetative and generative growth until the harvesting period.

Observation of Qualitative and Quantitative Characters of the Br 23 Banana Mutant Line

Agronomic characteristic observations during generative growth were divided into two groups, i.e., qualitative and quantitative parameters. All parameters are presented in Table 1 and Table 2.

Table 1. Observation of qualitative characters derived from Br 23 banana mutant line

No.	Characters	Observation of Br 23 mutant line		Description	cv. Pisang Ambon kuning
		Year 1	Year 2		
1	Variety groups	Clone	Clone	Clone	Clone
2	Cross section of pseudostem	Round	Round	Round	Round
3	Pseudostem color	Strong yellowish green (RHS 141,C), dark greyish purple (RHS 92,A)	Strong yellowish green (RHS 141,C), dark greyish purple (RHS 92,A)	Strong yellowish green with dark greyish purple	Green with blackish purple spot
4	Leaf shape	Lanset	Lanset	Lanset	Lanset
5	Upper leaf color	Greyish olive green (RHS 137,B)	Greyish olive green (RHS 137,B)	Greyish olive green	Green
	Lower leaf color	Whitish green (RHS 139,C)	Whitish green (RHS 139,C)	Whitish green	Whitish green
6	Cross section of leaf stalk No.3	Open canal, edge extend to the side	Open canal, edge extend to the side	Open canal, edge extend to the side	Open canal, edge extend to the side
7	Heart shape	Like a spear	Like a spear	Like a spear	Like a spear
8	Color of heart	Greyish reddish (RHS N77,C)	Greyish reddish (RHS N77,C)	Greyish reddish	Reddish purple
9	Fruit shape	Straight slightly curved	Straight slightly curved	Straight slightly curved	Curved
10	Fruit Color				
	- Young	Strong yellowish green (RHS 141,C)	Strong yellowish green (RHS 141,C)	Strong yellowish green	Green
	- Mature	Light yellow (RHS 11,B)	Light yellow (RHS 11,B)	Light yellow	Yellow
11	The Color of fruits flesh	Pale yellow (RHS 11,C)	Pale yellow RHS 11,C)	Pale yellow	Pale yellow
12	Cross section shape of fruits	Round	Round	Round	Round
13	Fruits taste	Sweet acidity	Sweet acidity	Sweet acidity	Sweet
14	Aroma	Fragrance	Fragrance	Fragrance	Fragrance

Vegetative Compatibility Group Test for Tropical Race 4 (TR4)

Vegetative compatibility group (VCG) tests for banana plant sensitivity to *Fusarium* wilt fungi were carried out at the Center for Tropical Fruit Research, Solok, West Sumatera on 2016-2017. Pseudostem from the infected sensitive plants that were previously planted on the *Fusarium* hot spot area at Pasar Jumat, South Jakarta was then taken for VCG analysis.

Isolation and Purification of Foc Isolates

The dried pseudostem of banana was cut into small pieces (0.5-1 cm) and then grown on a medium of 1/3 the composition of potato dextrose agar (PDA) containing 50 ppm streptomycin. The culture was incubated at room temperature for 2-4 days. *F. oxysporum*, which grew on the cultures, was characterized by the color of pink/purple colonies. The morphological identifications of growing colony of Foc was carried out under a microscope. The thin-walled macroconidia with 3-4 septa were generally found in conidiophores branches. Meanwhile, microconidia were oval or kidney in shape and short stem conidiophores were observed. Furthermore, isolates were purified in a single spore technique (SST) (Krnjaja et al., 2013).

Table 2. Observation of quantitative characters of Br 23 mutant line at field trial, Pasar Jumat-South Jakarta

No.	Plant Characters	Observation of agronomic characters from Br 23 mutant line			cv. Pisang Ambon kuning (control)
		Observation of the first year	Observation of the second year	Range of numbers from the first to the second year	
1	Plant height (cm)	182-220	198-225	182-225	350-400
2	Diameter of pseudostem (cm)	14-16	14-17	14-17	22.3-27.1
3	Leaf length (cm)	175-199	175-196	175-199	283-330
4	Leaf width(cm)	68-80	68-79	68-80	70-98
5	Ratio leaf length/Leaf width	2.48-2.57	2.48-2.57	2.48-2.57	3.36-4.04
6	Male flower length (cm)	23-27	23-27	23-27	55-60
7	Male flower width (cm)	8.5-9.5	8.5-9.0	8.5-9.5	43.96-56.52
8	The age of male flower after planting (months)	9-11	9-11	9-11	9-11
9	Harvested (months)	13-15	13-15	13-15	14-16
10	Fruit length (cm)	16.0-20	15-20	15-20	18.5-26.3
11	Thickness of fruit skin (mm)	3-4	3-4	3-4	2.6-4.5
12	Soluble Carbohydrate (°Brix)	25.05	19.80	19.80-25.05	15.5
13	Vitamin C content (mg/100 g)	29.66	38.89	29.66-38.89	11.23
14	Water content (%)	69.5-74.0	67-74	67-74	66.29
15	Protein content (%))	1.33	1.64	1.33-1.64	1.09
16	Sucrose (%)	25.04	23.5	23.5-25.04	ND*
17	Number of fruits/comb	14-18	14-17	14-18	13-26
18	Fruit weight/comb (kg)	1.5-2.3	1.1-2.3	1.1-2.3	3.8-8.6
19	Individual fruit weight (g)	110-150.8	94.0-157.6	94.0-157.6	185.3-300.1
20	Number of combs per bunch	8-11	8-11	8-11	6-9
21	Fruit weight per bunch (kg)	15.3-18.3	11-21.8	11-21.8	23-26

Remarks: * Note determined

Growth of Nit-mutants and Foc

VCG test was performed according to Dita et al. (2010). Pure Foc isolates from 7-day-old SST cultures on PDA media were planted on media potassium chlorate (MPK) media. Within 5-12 days, the sector appeared at the end of the colony, which marked that nit-mutants were formed. If nit-mutants were not formed within 5-12 days, repetition would be carried out. The growing ends of the sectors on the MPK media were then cut into small pieces (0.5 cm) and later transferred into a minimum media (MM). The tester materials were obtained from the Department of Primary Industry, Plant Pathology Section, Indooroopilly, Australia, which nit-mutants were known for their VCG codes. There were 17 testers used in this study, i.e., VCG 0120, VCG 0120/15, VCG 0121, VCG 0123, VCG 0124, VCG 0125, VCG 0124/5, VCG 0126, VCG 0128, VCG 0129, VCG 01211, VCG 01213, VCG 01216, VCG 01213/16, VCG 01218, VCG 01219, and VCG 01220. The tester of storage media was re-cultured on MM media for rejuvenation, then labeled according to the tester code.

Vegetative Compatibility Group Analysis (VCG)

The media used in the VCG analysis was minimum media (MM). During the test, each nit-mutant of the tested isolate was paired with all nit-mutants known to its VCG or tester. The rejuvenated tester in MM was cut into small pieces (0.5 cm) and then moved into the new MM and placed in the center of the petri dish. Furthermore, nit-mutant test isolates from MM were also cut into small pieces (0.5 cm) and placed as many as three pieces (as replications) around the tester (triangle formation). The inoculant were incubated at room temperature for 7-15 days. The pair of colonies planted in MM testing, and if they could form heterokaryons then it was compatible with the tester. Heterocaryon in the media were identified as visible white thickened hyphae between the tester and nit-mutant test isolates. The couples that were unable to form heterokaryons were considered incompatible. The compatibility or incompatibility showed that the isolates were identical or not identical to the tester.

Determination of Vitamin C, Protein, and Carbohydrates Content

The analysis of chemical contents of banana fruits, such as vitamin C and protein was conducted at the Laboratory of the Center for Agricultural Postharvest Research and Development. Meanwhile, the carbohydrate analysis was carried out at the Tropical Biology Chemistry Laboratory, Bogor.

Determination of Sucrose Content

The sucrose content of banana fruit was determined at the Plant Breeding Laboratory, Center for Application of Isotope and Radiation using refractometer PAL-1 equipment. Approximately, 50 g of banana fruits were blended to obtain smooth pulp, and then subsequently, it was taken into a sample weighing about 10 g. The sample pulp was later transferred into a 10 ml conical tube and centrifuged at 4000 rpm for 10 minutes. The supernatant was taken for around 300 µl, and then the absorbance was measured using a PAL-1 refractometer with three replications. For sample measurement, the standard solution was made of pure sucrose dissolved in sterile distilled water with the ratio of sucrose and water of 1:1, 1:2, 1:3, and 1:4 to obtain a standard curve.

Organoleptic Test

Banana aroma and taste test was carried out by distributing samples of ripe bananas and questionnaires to the respondents. After they tasted the fruit, they were asked to select one out of the multiple choice from the questions in the questionnaires. The answers consisted of a score ranging from 3+ (sweet taste), 2+ (less sweet), and 1+ (not sweet), while the score for the banana aroma was the same as the taste test.

RESULTS AND DISCUSSION

Vegetative Propagation of Br 23 Banana Mutant Line

From the observation results, it showed that new shoots emerged from the lateral part of the explant after four to six weeks culture on MS media. Plantlets grew and developed during four weeks in culture media. At this time, the plantlets were transferred to the rooting media. The observation showed that the roots grew perfectly after one month on rooting media. The plantlets with sufficient roots were then acclimatized in a greenhouse before being transferred to a polybag. During acclimatization, the plantlets were covered with transparent plastics to maintain their moisture. The plantlet acclimatization process in the greenhouse is explained below.

Plants Acclimatization and Growth

The observation results showed that new leaves started to grow at two or three weeks after the plantlet was transferred to a polybag. During

this period, leaves that might appeared wilted in the first week began to look fresh, and then new leaves were developed. After acclimatization, the plant looked healthy. At this time, the plants were moved to a 1 kg polybag to make the plant growth more robust and not stress. After one month, the plants grown in a small polybag were then transferred to a 5 kg polybag filled with mixture of compost and soil (1:1 v/v). In the second stage of the plant transfer, plant growth was more apparent and progressed very rapidly. After 2-3 months, the plants were ready to be moved to the field that had been prepared previously. Thirty plants of Br 23 mutant lines were planted in the field, arranged in 2 lines. Based on observation, it showed that the first banana flower began to emerge from one of the Br 23 banana mutant lines after six months after planting on the field. As the banana fruits began form from the flower, it was then covered by blue plastics in order to obtain smooth fruit peel and avoid pest attacks.

Morphological Description

Detailed morphological descriptions of the Br 23 mutant line can be seen in the pictures shown in Table 1. The description of the Br 23 mutant lines was carried out according to Simmonds & Shepherd (1955). Based on the description, Br 23 banana mutant line was classified in the acuminata group, which has an AAA genome.

Sixteen morphological characters of the Br 23 banana mutant line were observed during vegetative and generative growth. Observation results showed the differences with other mutant lines, i.e., the fruit stalk was shorter compared to Br 09 mutant line (data was not shown). This Br 09 banana mutant line was derived from the same parent, i.e., pisang ambon. The length ratio of the X-axis and the Y-axis of the banana heart of the Br 23 mutant line was <0.25. Observation on the ratio of width and length of the heart was done in 2-3 days after the banana heart emerged. Meanwhile, the observation of the length ratio of the heart in the cavendish banana group showed the same result (data are not shown here). This ratio showed that the Br 23 banana mutant line belonged to a banana group with AAA genome with the same morphology. According to Simmonds & Shepherd (1955), the shape of the banana heart and the ratio of all *Musa acuminata* groups had an x/y ratio of <0.28 and >0.30 for *Musa balbisiana*.

Qualitative and Quantitative Observations of Br 23 Banana Mutant Line

The observation results showed that quantitative characters of the Br 23 mutant line, such as the weight and size of the banana, were different during the rainy and dry seasons. During dry season (May–August), the weight and size of fruit harvested were slightly lower compared to that of the rainy season. Meanwhile, the qualitative characters of the Br 23 banana mutant line, such as leaf and fruit color when young and ripe, heart color and shape were also observed. The observation results of the mutant line are presented in Table 1. These color observations were standardized using RHS color chart (sixth ed. 2015). Observations of banana plant morphological characters, such as leaf, stem, heart, male flower, fruit, and fruit stalk are also presented in Table 3. Banana mutant line Br 23 that was derived from irradiated pisang ambon, is belonged to *Musa acuminata* group. The morphological descriptions are in accordance with those proposed by the World Taxonomist for Bananas of the AAA genome.

The observation of the quantitative characters showed that the plant height of the Br 23 mutant line was shorter compared to the pisang ambon kuning cultivar, which was used as the control. Meanwhile, the vitamin C content in the Br 23 banana mutant line was considered high (Table 2).

Vitamin C, Protein, and Carbohydrate Content of Br 23 Mutant Line

Most pisang ambon in Indonesia has vitamin C content ranging from 11 to 15 mg/100 g of fruit and is sensitive to *Fusarium* wilt. However, Br 23 mutant line contains 29.89 to 38.89 mg/100 g of vitamin C, which is higher compared to their sister line that contains only 9 to 10 mg/100 g (Table 2). Thus, by planting the Br 23 banana mutant lines, farmers in rural communities will provide a sustainable source of vitamin C. The high content of vitamin C in banana fruit provisions people living in a rural areas the need of vitamin C. In addition, Br 23 mutant lines are tolerant to *Fusarium* wilt disease. Therefore, the farmer cultivating Br 23 banana mutant line gain several advantages, such as its tolerance against *Fusarium* wilt. Meanwhile, protein and carbohydrate content in the fruit accounts for 1.33-1.64% and 19.80-25.05%, respectively. These amounts are not significantly different in comparison to pisang ambon kuning (Table 2).

Table 3. Observation of morphological performance from Br 23 banana mutant line

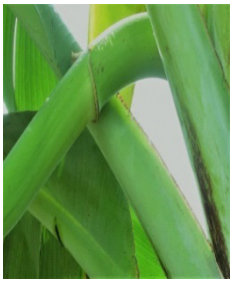
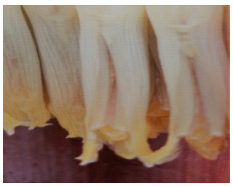
No.	Plant characters	Morphology	Note
1	Pseudostem color		Strong yellowish green (RHS 141,C) with dark greyish purple (RHS N92,A)
2	Leaf base		Leaf base was not simetric with strong purplish red (RHS 63,B)
3	Petiolar canal		Open
4	Peduncle		Peduncel with hair and ±32 cm length
5	Pedicels		Short
6	Ovules		Light greenish yellow (RHS 1,C)

Table 3. *(continued)*

No.	Plant characters	Morphology	Note
7	Bract shoulder		X = width, X-axis Y = length , Y-axis Ratio: x/y = 0.20
8	Bract curling		Curling
9	Bract shape		*Lanceolatus*
10	Bract apex		Tapered
11	Bract color		Color: Greyish reddish purple (RHS N77,C)
12	Color fading		Yellowish white (RHS 158,C) mixed with strong purplish red (RHS 60,d)
13	Bract scars		ProtruGrede

Table 3. *(continued)*

No.	Plant characters	Morphology	Note
14	Free tepal of male Flower color		Light cream (RHS 155,C)
15	Male Flower Color		Greenish white (RHS 155,C)
16	Stigma Color		Brilliant yellow (RHS 10,A)

Remarks: Morphological characters observation based on Simmonds & Shepherd (1955); Color standard used color chart from Royal Horticultural Society (RHS), sixth edition (2015)

Organoleptic Test Result

The result of the organoleptic test showed that all pisang ambon mutants had fragrance. Their fruit tasted sweet to sweet acid. It could be due to the high content of vitamin C in the fruit.

Vegetative Compatibility Test of TR4 Isolates of *Fusarium*

Vegetative compatibility groups (VCG) have been used to identify the differences among *Fusarium* isolates. Recent reports mentioned that a new strain of *Fusarium* derived from race 4 called TR4 (tropical race 4) attacked cavendish subgroups (grande naine and other *Musa acuminate* (AAA genome)) (Dita et al., 2010). According to Ordonez et al. (2015), TR4 represents VCG01213/16, the strain that has detrimental effect on bananas with AAA genomes, such as cavendish, pisang ambon, and barangan in Indonesia. The mutation breeding approach to obtain the resistance cultivar of pisang ambon has been carried out in our laboratory since 2004. Six putative mutant lines were obtained during the observation at the hot spot area of *Fusarium*, which has been used continuously to grow banana plants in the field. Sensitive cultivars, such as pisang ambon kuning and pisang barangan were also grown at these *Fusarium* hot spot areas, and used for sensitive control to the disease. Several pisang ambon and barangan plants attacked by Fusarium wilt were taken as samples. Later, the fibers of their pseudostems were used for VCG test at the Fruit Research Center Laboratory, Solok, West Sumatera. The results of the VCG test showed that mutant lines (AAA), such as BSF.1 and BSF.4 (pisang barangan), and Br 0 and Br 22 (pisang ambon) mutant lines were indeed attacked by *Fusarium* wilt (Table 4). Both Br 12 and Br 11 banana mutant lines showed similar symptom with *Fusarium* wilt. Nevertheless, the VCG test did not show nit mutant. BSF.1, BSF.2, and BSF.4 derived from irradiated pisang Barangan cultivar, whereas Br 03, Br 0, Br 11, and Br 12 mutant lines were derived from pisang ambon cultivar. Banana mutant lines derived from pisang barangan were more sensitive when compared to mutants of pisang ambon, such as Br 11 and Br 12 because BSF mutant line has been attacked since they reached 3 to 4 months old in the field.

The symptoms of *Fusarium* wilt in banana plants are indicated by the yellowing leaves and then spreading to the younger leaves. Later, the

leaves will wither and dry out. Mintoff et al. (2021) mentioned several FHIA parental lines and hybrids, the cavendish (AAA) selections GCTCV 215 and GCTCV 247 from TBRI and an Indonesian selection CJ19 showed either very little to no occurrence of plant death due to the disease. The identification of transgenic cavendish with resistance to TR4 showed that transgene expression in the RGA2 lines is strongly correlated with the resistance (Dale et al., 2017).

The evolution of diploid *Musa acuminata* to triploid can be explained by spontaneous mutation theory. It is likely to occur in nature due to plants being bombarded by continuous ultraviolet rays from sunlight. Ultraviolet has a short wavelength sufficient to penetrate plant cells and results in spontaneous mutations on the genomic DNA chain. Chromosomal mutations can lead to polyploidy, such as triploid and tetraploid. The origin of triploid banana from *Musa acuminata* (A-genome) and *Musa balbisiana* (B-genome) is based on the exchange that frequently occurs between A- and B-subgenomes in allopolyploids (Wang et al., 2019). While Martin et al. (2017) mentioned that triploid cultivars were caused by the rearranged chromosome structure, such as those found in wild *Malaccensis* ssp. accessions. It is thus suggested that this rearrangement occurs in *M. acuminata* ssp. Malaccensis. Several types of edible banana cultivars, such as diploid, triploid, or tetraploid hybrids, originate from natural cross-hybridization between subspecies of diploid *Musa acuminata* or between *M. acuminata* and diploid *Musa balbisiana* (Šimoníková et al., 2020).

Furthermore, gamma rays can accelerate mutations in plant cells, both vegetative and generative cells. Gamma rays have deep penetrating power to plant cells, resulting in changes in genetic structures in the plant genome through the mutation process. According to Alpen (1997), four events occur during the treatment of physical mutagen substances to plant cells, namely: 1) Physical process, that is, the occurrence of the photon energy transfer from the source to plant cells, resulting in a chemical process in cells; 2) Chemical process, the energy received by plant cells from energy sources (gamma rays) results in water radiolysis in the cells. As a result of this radiolysis, free radicals are formed in the cells and result in changes in the bonds in the DNA base or base structure. As such, the third phase will take place, which is called biological process of the occurrence of constitutional genetic changes in a DNA chain in the nuclear genome or the mitochondria genome. Then, the cell performs self-improvement called the biochemical process. DNA polymerase plays a vital role in repairing the DNA chain in this biochemical or enzymatic process. During the repairing process, several DNA bases in a DNA chain change from their origin, which is known as mutations.

Table 4. VCG test for several mutant lines attacked by Fusarium wilt at experimental field of banana, South Jakarta

No.	Plants attacked by Fusarium wilt the same location with Br 23 banana mutant	Compatibilitas (Isolate) used for VCG test	Tropical Race 4
1	BR0	01213/16	Race 4 (TR4)
2	BSF1	01213/16	Race 4 (TR4)
3	BR22	01213/16	Race 4 (TR4)
5	BSF4	01213/16	Race 4 (TR4)
6	BR12	No, nit Mutant	-
7	BSF2	01213/16	Race 4 (TR4)
8	BR.11	No, nit mutant	-
9	BR03	01213/16	Race 4 (TR4)

The use of gamma radiation for plant breeding, especially those vegetatively propagated plants, such as bananas, is efficacious because most banana plants are triploid (AAA) and parthenocarpy. It is not possible to improve genetic traits through conventional breeding to obtain superior alleles with desirable traits, such as tolerance to *Fusarium* wilt. Several reports mentioned that gamma radiation (Co-60) has been effective in creating genetic variability in bananas. In addition, the generated mutants have indicated tolerance to banana bunchy top virus (BBTV) (El-Sayed et al., 2011). Similarly, Chen et al., (2013) reported that EMS-induced mutation in banana-generated putative mutants, which showed tolerance to Foc. Meanwhile, Li et al., (2020) developed banana breeding using physical and chemical mutagenesis. They successfully obtained 39 mutant lines with moderate resistance to *Fusarium* advanced tools, such as CRISPR/ Cas-based genome that could be used as the most powerful tool for developing against bacterial pathogens resistant crop varieties (Tripathi et al., 2022). Besides, spontaneous mutation on plantlets during plant tissue culture for micropropagation can be a source of genetic variation called somaclonal variation. This spontaneous mutation can produce mutants that show tolerance to *Fusarium* wilt in the cavendish cultivar (Hwang & Ko 2004; Viljoen et al. 2020). According to Siamak & Zheng (2018), apart from in vitro mutation and somaclonal variation, somatic hybridization can improve breeding program-related *Fusarium* resistance in bananas.

According to Rocha et al. (2021), the fungus *Fusarium oxysporum* f. sp. Cubense (Foc), tropical race 4 (TR4), causes *Fusarium* wilt in bananas, which threatens the fruit cultivation and trading disruption. Producing banana mutants that are tolerant to *Fusarium* wilt provides an added value to the banana farmers because they do not have to worry about the devastation of their banana plants caused by *Fusarium* wilt. Experience so far has shown that *Fusarium* wilt has destroyed banana plantations in both small and large scales in Indonesia.

Dale et al. (2017) mentioned that transgene expression in the RGA2 lines was strongly correlated with the disease-resistance. Endogenous Resistance Gene Analog2 (RGA2) homologs are also present in cavendish but are expressed tenfold lower than that in their most resistant transgenic line. Meanwhile, on the susceptible cv. Williams, no increase in callose content is observed. Based on supporting data showed that RGA2 could be a key factor involved in both R1 and TR4 resistance, whereaas TR4 development depend on activating resistance to Race 1. (García-Bastidas et al., 2022).

CONCLUSION

Analysis of the morphological character of the Br 23 banana mutant line indicates that it strongly belongs to the acuminata group. Beside the high content of vitamin C of the fruit, this Br 23 mutant line also has *Fusarium* wilt-tolerant for tropical race 4 (TR4) fungi. Therefore, in addition to carbohydrate provision for the community, the spread and cultivating Br 23 banana cultivar in rural areas will help prevent vitamin C-deficiency.

ACKNOWLEDGEMENT

The authors would like to thank the Indonesian government for providing research grant. The authors are also grateful to Dr. Sasanti Widiarsih for critical reading of this manuscripts.

REFERENCES

Alpen, E. L. (1997). *Radiation Biophysics* (2nd ed.). Academic Press.

Chen, Y. F., Chen, W., Huang, X., Hu, X., Zhao, J. T., Gong, Q., Li, X. J., & Huang, X. L. (2013). Fusarium wilt-resistant lines of Brazil banana (*Musa* spp., AAA) obtained by EMS-induced mutation in a micro-cross-section cultural system. *Plant Pathology*, *62*(1), 112–119. https://doi.org/10.1111/j.1365-3059.2012.02620.x

Dale, J., James, A., Paul, J.-Y., Khanna, H., Smith, M., Peraza-Echeverria, S., Garcia-Bastidas, F., Kema, G., Waterhouse, P., Mengersen, K., & Harding, R. (2017). Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nature Communications*, *8*(1), 1496. https://doi.org/10.1038/s41467-017-01670-6

Dita, M. A., Waalwijk, C., Buddenhagen, I. W., Souza Jr, M. T., & Kema, G. H. J. (2010). A molecular diagnostic for tropical race 4 of the banana fusarium wilt pathogen. *Plant Pathology*, *59*(2), 348–357. https://doi.org/10.1111/j.1365-3059.2009.02221.x

Dita, M., Barquero, M., Heck, D., Mizubuti, E. S. G., & Staver, C. P. (2018). Fusarium Wilt of banana: Current knowledge on epidemiology and research needs toward sustainable disease

management. *Frontiers in Plant Science*, *9*, 1468. https://doi.org/10.3389/fpls.2018.01468

El-Sayed, E., Mahfouze, S., Shaltout, A., El-Dougdoug, K., & Sayed, R. (2011). Mutation breeding of banana cv. 'Grand-Nain' for resistance to some banana viruses using biotechnology and physical mutagens. *The African Journal of Plant Science and Biotechnology, 5*(1), 35-40. http://www.globalsciencebooks.info/Online/GSBOnline/images/2011/AJPSB_5%281%29/AJPSB_5%281%2935-40o.pdf

García-Bastidas, F. A., Arango-Isaza, R., Rodriguez-Cabal, H. A., Seidl, M. F., Cappadona, G., Segura, R., Salacinas, M., & Kema, G. H. J. (2022). Induced resistance to Fusarium wilt of banana caused by Tropical Race 4 in Cavendish cv Grand Naine bananas after challenging with avirulent Fusarium spp. *PLOS ONE*, *17*(9), e0273335. https://doi.org/10.1371/journal.pone.0273335

Hwang, S.-C., & Ko, W.-H. (2004). Cavendish Banana Cultivars Resistant to Fusarium Wilt Acquired through Somaclonal Variation in Taiwan. *Plant Disease*, *88*(6), 580–588. https://doi.org/10.1094/PDIS.2004.88.6.580

Krnjaja, V., Levic, J., Stankovic, S., & Vasic, T. (2013). The use of vegetative compatibility tests for identification of biodiversity of phytopathogenic fungi. *Pesticidi i Fitomedicina*, *28*(3), 157–165. https://doi.org/10.2298/PIF1303157K

Li, C., Deng, G., Yang, J., Viljoen, A., Jin, Y., Kuang, R., Zuo, C., Lv, Z., Yang, Q., Sheng, O., Wei, Y., Hu, C., Dong, T., & Yi, G. (2012). Transcriptome profiling of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum* f. Sp. Cubense tropical race 4. *BMC Genomics*, *13*(1), 374. https://doi.org/10.1186/1471-2164-13-374

Li, C. Y., Yi, G. J., Xiang, D., Hu, C. H., Yang, Q. S., Bi, F. C., Gao, H., Sheng, O., Deng, G. M., Dong, T., & Dou, T. X. (2020). A comprehensive strategy for managing Fusarium wilt of banana. *Acta Horticulturae*, *1272*, 67–72. https://doi.org/10.17660/ActaHortic.2020.1272.9

López, J., Rayas, A., Santos, A., Medero, V., Beovides, Y., & Basail, M. (2017). Mutation Induction Using Gamma Irradiation and Embryogenic Cell Suspensions in Plantain (Musa spp.). In J. Jankowicz-Cieslak, T. H. Tai, J. Kumlehn, & B. J. Till (Eds.), *Biotechnologies for Plant Mutation Breeding* (pp. 55–71). Springer International Publishing. https://doi.org/10.1007/978-3-319-45021-6_4

Martin, G., Carreel, F., Coriton, O., Hervouet, C., Cardi, C., Derouault, P., Roques, D., Salmon, F., Rouard, M., Sardos, J., Labadie, K., Baurens, F.-C., & D'Hont, A. (2017). Evolution of the Banana Genome (*Musa acuminata*) Is Impacted by Large Chromosomal Translocations. *Molecular Biology and Evolution*, *34*(9), 2140–2152. https://doi.org/10.1093/molbev/msx164

Maryani, N., Lombard, L., Poerba, Y. S., Subandiyah, S., Crous, P. W., & Kema, G. H. J. (2019). Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. Sp. Cubense in the Indonesian centre of origin. *Studies in Mycology*, *92*, 155–194. https://doi.org/10.1016/j.simyco.2018.06.003

Mintoff, S. J. L., Nguyen, T. V., Kelly, C., Cullen, S., Hearnden, M., Williams, R., Daniells, J. W., & Tran-Nguyen, L. T. T. (2021). Banana Cultivar Field Screening for Resistance to Fusarium oxysporum f.sp. Cubense Tropical Race 4 in the Northern Territory. *Journal of Fungi*, *7*(8), 627. https://doi.org/10.3390/jof7080627

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Olivares, B. O., Rey, J. C., Lobo, D., Navas-Cortés, J. A., Gómez, J. A., & Landa, B. B. (2021). Fusarium Wilt of Bananas: A Review of Agro-Environmental Factors in the Venezuelan Production System Affecting Its Development. *Agronomy*, *11*(5), 986. https://doi.org/10.3390/agronomy11050986

Ordonez, N., Seidl, M. F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B. P. H. J., Ploetz, R. C., & Kema, G. H. J. (2015). Worse Comes to Worst: Bananas and Panama Disease—When Plant and Pathogen Clones Meet. *PLOS Pathogens*, *11*(11), e1005197. https://doi.org/10.1371/journal.ppat.1005197

Pegg, K. G., Coates, L. M., O'Neill, W. T., & Turner, D. W. (2019). The Epidemiology of Fusarium Wilt of Banana. *Frontiers in Plant Science*, *10*, 1395. https://doi.org/10.3389/fpls.2019.01395

Ploetz, R. C. (2015). Fusarium Wilt of Banana. *Phytopathology®*, *105*(12), 1512–1521. https://doi.org/10.1094/PHYTO-04-15-0101-RVW

Rocha, A. D. J., Soares, J. M. D. S., Nascimento, F. D. S., Santos, A. S., Amorim, V. B. D. O., Ferreira,

C. F., Haddad, F., Santos-Serejo, J. A. D., & Amorim, E. P. (2021). Improvements in the Resistance of the Banana Species to Fusarium Wilt: A Systematic Review of Methods and Perspectives. *Journal of Fungi*, *7*(4), 249. https://doi.org/10.3390/jof7040249

Rohmah, Y. (2016). *Outlook: Banana commodity*. Center for Agricultural Data and Information Systems Ministry of Agriculture.

Royal Horticultural Society (RHS) Colour Chart (2015) Revised edition, https://labtekservices.co.uk/product/rhs-large-colour-chart-sixth-revised-edition/

Siamak, S. B., & Zheng, S. (2018). Banana Fusarium wilt (*Fusarium oxysporum* f. Sp. Cubense) control and resistance, in the context of developing wilt-resistant bananas within sustainable production systems. *Horticultural Plant Journal*, *4*(5), 208–218. https://doi.org/10.1016/j.hpj.2018.08.001

Simmonds, N. W., & Shepherd, K. (1955). The taxonomy and origins of the cultivated bananas. *Journal of the Linnean Society of London, Botany*, *55*(359), 302–312. https://doi.org/10.1111/j.1095-8339.1955.tb00015.x

Šimoníková, D., Němečková, A., Čížková, J., Brown, A., Swennen, R., Doležel, J., & Hřibová, E. (2020). Chromosome Painting in Cultivated Bananas and Their Wild Relatives (Musa spp.) Reveals Differences in Chromosome Structure. *International Journal of Molecular Sciences*, *21*(21), 7915. https://doi.org/10.3390/ijms21217915

Sisodia, A., & Singh, A. K. (2015). Studies on gamma ray induced mutants in gladiolus. *The Indian Journal of Agricultural Sciences*, *85*(1), 79–86. https://doi.org/10.56093/ijas.v85i1.46003

Tripathi, L., Ntui, V. O., & Tripathi, J. N. (2022). Control of Bacterial Diseases of Banana Using CRISPR/Cas-Based Gene Editing. *International Journal of Molecular Sciences*, *23*(7), 3619. https://doi.org/10.3390/ijms23073619

Viljoen, A., Mostert, D., Chiconela, T., Beukes, I., Fraser, C., Dwyer, J., Murray, H., Amisse, J., Matabuana, E. L., Tazan, G., Amugoli, O. M., Mondjana, A., Vaz, A., Pretorius, A., Bothma, S., Rose, L. J., Beed, F., Dusunceli, F., Chao, C.-P., & Molina, A. (2020). Occurrence and spread of the banana fungus *Fusarium oxysporum* f. Sp. Cubense TR4 in Mozambique. *South African Journal of Science*, *116*(11/12). https://doi.org/10.17159/sajs.2020/8608

Wang, Z., Miao, H., Liu, J., Xu, B., Yao, X., Xu, C., Zhao, S., Fang, X., Jia, C., Wang, J., Zhang, J., Li, J., Xu, Y., Wang, J., Ma, W., Wu, Z., Yu, L., Yang, Y., Liu, C., … Jin, Z. (2019). *Musa balbisiana* genome reveals subgenome evolution and functional divergence. *Nature Plants*, *5*(8), 810–821. https://doi.org/10.1038/s41477-019-0452-6

Wang, Z., Zhang, J., Jia, C., Liu, J., Li, Y., Yin, X., Xu, B., & Jin, Z. (2012). De Novo characterization of the banana root transcriptome and analysis of gene expression under *Fusarium oxysporum* f. Sp. Cubense tropical race 4 infection. *BMC Genomics*, *13*(1), 650. https://doi.org/10.1186/1471-2164-13-650

Yamaguchi, H. (2018). Mutation breeding of ornamental plants using ion beams. *Breeding Science*, *68*(1), 71–78. https://doi.org/10.1270/jsbbs.17086

Yusnita, Danial, E., & Hapsoro, D. H. (2015). In vitro shoot regeneration of Indonesian bananas (*Musa* spp .) cv. ambon kuning and raja bulu, plantlet acclimatizationand field performance. *AGRIVITA*, *37*(1), 51–58. http://dx.doi.org/10.17503/Agrivita-2015-37-1-p051-058