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MORPHOLOGY AND REPRODUCTIVE FUNCTION OF INDUCED AUTOTETRAPLOID BANANA BY CHROMOSOME DOUBLING

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SUMMARY

Triploid bananas can be generated by crossing between diploid and tetraploid banana cultivars. The production of tetraploid banana plants as candidate parents can be obtained by the application of oryzalin or colchicines. The objectives of this study were to characterize the autotetraploid banana plant "Pisang Madu" (*Musa,* AAAA) induced by in-vitro oryzalin treatment and to use these tetraploid plants as 2x gamete donors in crosses with diploids to generate triploid hybrids. In-vitro shoot cultures of parthenocarpic diploid banana "Pisang Madu" (*Musa*, AA) were initiated and the in vitro shoots were treated by oryzalin at a concentration of 60µM for 7 days in a liquid MS basal medium with addition of 2 mg/l BA. The treated shoots were selected for the solid tetraploids using flowcytometer. Morphology characterization and was study of reproductive function conducted at the Cibinong Science Center for 2 cycles. Fifty two quantitative and qualitative characters were recorded based on UPOV for Banana. Compared with the original diploids, the autotetraploid bananas showed an increase in fruit size and bunch weight. They also showed drooping leaves and truncated fruit apex. Furthermore, the autotetraploids were successfully used as 2x gamete donors in generating triploid hybrids.

Keywords: Autotetraploid banana, chromosome doubling, *Musa,* oryzalin, triploid hybrids

Key findings: Autotetraploid banana was obtained by treating in vitro shoots of diploid banana with oryzalin. The autotetraploids showed increased fruit size and bunch weight. They were successfully used as 2x gamete donors in generating secondary triploid hybrids.

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INTRODUCTION

Banana (*Musa* spp.) is the most important and widely planted fruit in Indonesia. Indonesia is the center of origin and diversity of banana (Nasution, 1991; Perrier *et al.,* 2011). There are at least 15 species of *Musa,* 15 varieties of *M. acuminata* Colla, three varieties of *M. balbisiana* Colla, and more than 200 local cultivars (Nasution, 1991). However, most of these banana cultivars have not been improved by breeding activities. The genetic complex of banana (parthenocarpic, asexual behavior of edible clones, sterility, and polyploidy) and a lack of knowledge in genetics and the type of inheritance of traits limits the progress of the banana breeding program in the country (Sasson, 1997; Amaral *et al.,* 2015).

Breeding methods used in banana improvement are performed on the following crosses of: diploids x diploids, triploids xdiploids, and tetraploidsxdiploids to generate improved banana population from which superior hybrids are selected in terms of bunch yield, resistance to pests and diseases, and fruit quality and then evaluated for subsequent breeding activities (Oselebe *et al*., 2006; Wilberforce *et al*., 2014). Unfortunately, the availability of 2n gamete is rare and unstable in banana. Therefore, the production of fertile autotetraploid plants is crucial and will enhance the production of triploid hybrids when they directly crossed with improved diploids from a large diploid germplasm (Silva *et al*., 2001; Bakry *et al*., 2007).

Efforts to produce more desirable genotypes by induced polyploidy has been conducted in bananas (Vakili, 1967; Stover and Buddenhagen, 1986; Hamill *et al*., 1992; Van Duren *et al*., 1996; Ganga and Chezhiyan, 2002; Bakry*et al*., 2007; Kanchanapoom and Koarapatchaikul, 2012, Amaral *et al*., 2015, Poerba *et al*., 2017, 2018). Chromosome doubling using antimitotic agents requires an efficient system of polyploidy induction as well as an effective method for ploidy verification. According to Dhooghe *et al*. (2009), oryzalin might be used at lower concentrations compared to colchicines and trifluralin. Therefore, cytotoxic side effects can be minimized. Oryzalin has been used in induction of tetraploids in banana (Van Duren *et al*., 1996; Ganga and Chezhiyan 2002; Kanchanapoon and Koarapatchaikul, 2012; Poerba *et al*, 2014, 2016, 2017, 2018).

Autotetraploids were confirmed to have an increased leaves and fruit size in comparison to the original diploids (Kanchanapoom and Koarapatchaikul, 2012; Poerba *et al*., 2017a, 2018). The autotetraploid PisangLilin had an increased plant height, number of leaves at flowering and harvest, pseudostem diameter, and fruit size and bunch (Amaral *et al*., 2015).

The cultivar used in this study, "Pisang Madu" (*Musa* AA), is a diploid local cultivar, parthenocarpic, tasty and sweet, small sized, and had finger drop longevity. This study was undertaken to develop and characterize autotetraploid banana induced by in-vitro oryzalin treatment and to be used in crosses as 2n gamete donor to generate secondary triploid hybrids.

MATERIALS AND METHODS

Plant materials and shoot cultures

Diploid banana "Pisang Madu" (*Musa,*AA) was accessed from ITC 0258. Shoot cultures were established and multiplied in MS medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, 100 mg/Lmyo inositol, 2 mg/L BA and solidified by 7 g/L agar. The media were adjusted to pH of 5.7-5.8 and the cultures were maintained at 25°C.

Induction of autotetraploidy

Induction of autotetraploidy was conducted using oryzalin as described by Van Duren *et al*. (1996). The shoots were extracted from the medium, and were treated with the antimitotic agent oryzalin at concentration of 60µM for seven days in liquid medium with agitation (60 rpm). After treatment, the shoots were washed three times with sterile distilled water and transferred to a proliferation medium for further culturing to reduce the frequency of mixoploids (plant material containing cells with varied chromosome number). The plants were then transferred to a rooting medium (MS supplemented by sucrose 30 g/L and solidified by 7g/L agar).The explants were kept in a room with a photoperiod of 16 hours, and a temperature of 25 ± 2°C during their growing phase. The cultures were sub-cultured for 5-6 times to separate the mixoploids. The plantlets were transferred to a greenhouse and placed in cultivation pots with a medium composed of sand, coco peat (coconut fiber), and soil compost (1:1:1), and were irrigated under 50% shading. After 60 days, the plants were transplanted to 20 L plastic polybags with the same medium composition. After 2 months, the banana plants were ready for field planting. All treated plants and control were planted in 5-plant rows using randomized complete block design.

Identification of ploidy level using flow cytometer

DNA content was analyzed by flow cytometry to determine the ploidy levels of regenerated plants. Ploidy determination was performed using a Partec PAS II flow cytometer (FCM) (Partec GmbH, Münster, Germany). Samples were prepared according to Doležel *et al*. (1994, 1997) with minor modification (Poerba *et al*., 2018). Approximately 20-30 mg of fresh leaf samples from cigar leaves (control and treated samples) were chopped with a sharp scalpel blade in a glass petri dish containing 1 ml of LB01 buffer (Doležel *et al*., 1989) of the following composition: 15 mM TRIS, 2mM Na2 EDTA, 80 mMKCl, 20 mMNaCl, 0.5 mMspermine, 15 mMmercaptoethanol, and 0.1% Triton X-100, with pH of 7.5. The buffer was supplemented with DAPI (4', 6-diamidino-2-phenylindole) at final concentration of 2 µg/ml to stain nuclear DNA. The suspension of released nuclei was filtered through a 50 µm nylon mesh and kept on ice before analysis. The relative DNA content of the sample was then determined using FCM analysis.

Relative DNA content is given in C units. The 1C value is DNA content of haploid set of chromosomes (n). The distribution of fluorescence intensities (relative DNA content) obtained after flow cytometric analysis is usually in arbitrary units (channel numbers). In this study, we used a

sample prepared from *Musa acuminata* ssp. m*alaccensis* (2n = 22) as a diploid reference (Poerba *et al*., 2018) and the flow cytometer was adjusted so that the peak representing its G1 nuclei appeared at channel 200. This setting was kept constant and other samples were characterized by the relative position of their G1 peak.

Characterization of plant morphology

Fifty two characters were evaluated for two reproductive cycles based on UPOV (2010).

Hybridization for evaluation of reproductive potential of autotetraploid bananas

Hybridization of autotetraploids x diploids and diploidsxautotetraploidshave been conducted. The autotetraploid banana and two wild banana varieties i.e., *Musa acuminata* Colla var. *malaccensis* (Ridl.)Nasution and *M. acuminata* Colla var. *zebrina* (v. Houtte) Nasution were used as parents for the hybridization. The flowers were pollinated and covered with plastic net. Seeds were collected from each cross pollinated fruit at maturity. The seeds were then separated from the pulp by continuous washing in tap water.

Washed seeds were transferred to a beaker with water for 15 minutes. Only the sunken seeds were used, since most of the floating seeds have either no endosperm or embryo. Seed disinfection was performed under sterile conditions in a laminar hood. Seeds were treated with 20% sodium hypochlorite for 15 minutes. Before and after each treatment, the seeds were rinsed with sterile distilled water for 2–3 times. Finally, the seeds were transferred to a sterile petri plate for embryo extraction. A longitudinal fissure was made in each seed and the whitish, mushroom-shaped embryo, was removed. The excised embryos were cultured in a medium consisting of Murashige and Skoog salts (1962), 30 g/L of sucrose, 100 mg/L myo inositol, 1 mg/L biotin, 0,5 mg/L BA, and the pH was adjusted to 5.8 and autoclaved at 121°C for 20 minutes. The embryo cultures were kept on dark until shoots were growing. The shoots were then transferred to a media containing a proliferation medium for multiplication (MS medium with sucrose 30 g/L, 100 mg/L myo inositol, 2 mg/L BA, and solidified with 7 g/L agar). The shoots were then transferred to a rooting medium MS free hormoneand solidified with 7 g/L agar. The explants were kept in a room with a photoperiod of 16 hours, and a temperature of 25 ± 2 °C during their growth phase. The plantlets (rooted plants) were transferred to a greenhouse and placed in cultivation pots with a medium composed ofsand, coco peat (coconut fiber), soil compost (1:1:1), and irrigated under 50% shading. After 60 days, the plants were transplanted to 20 L plastic polybags with the same medium composition. After 2 months, banana hybrid plants were ready for field planting. The ploidy levels of the hybrids were determined using flow cytometer.

RESULTS

Ploidy identification

Flow cytometry is increasingly employed as the method of choice for

determination of nuclear DNA content and ploidy level in plants because it provides exceptional rapidity, convenience, and accuracy. Flow cytometry was used on regenerated plants to give an accurate estimation of nuclear DNA content (Table 1). Figure 1 showed the result of flow cytometry measurement with three types of histograms. Control diploid banana containing 2C DNA showed peak at channel 200 (Figure 1. A), diploid treated banana containing 2C DNA showed peak at channel 200 (Figure 1.B), autotetraploid banana containing 4C DNA showed peak at channel 400 (Figure 1.D), and mixoploid banana containing 2C and 4C DNA showed peak at channel 200 and 400(Figure 1.C).

Table 1. Ploidy level of banana (control) and oryzaline-treated plants.

Sample No	Mean (Florescent density)	CV (%)	Ploidy	Sample No	Mean (Florescent density)	CV (%)	Ploidy
Musa acuminata var. *malaccensis*				11	384.62	10.03	4x
Standard	209.53	5.53	2x	12	403.02	7.69	4x
Banana cultivar (*Musa* AA)				13	422.67	7.65	4x
Control	216.32	6.93	2x	14	394.78	7.93	4x
Treated							
1	188.69	10.56	2x	15	398.03	8.74	4x
2	182.43	5.50	2x	16	392.75	8.59	4x
3	197.17	6.32	2x	17	383.64	8.96	4x
4	187.00	8.28	2x	18	386.94	6.39	4x
5	192.96	8.54	2x	19	397.42	9.01	4x
6	203.65	6.62	2x	20	391.70	8.53	4x
7	201.76	6.56	2x	21	395.63	6.88	4x
8	195.81	12.35	mix 2x-	22	425.52	6.32	4x
	380.37	8.62	4x	23	431.67	5.45	4x
9	215.81	7.39	mix 2x-	24	415.05	5.8	4x
	429.30	5.37	4x	25	422.98	5.75	4x
10	210.56	8.21	mix 2x-				
	424.49	5.82	4x				

Morphological characteristics of autotetraploid banana

The autotetraploids exhibited different morphological characteristics compared to the diploids, specifically in the number of suckers, pseudostem and leaf size, plant habit, the compactness of bunch, fruit size and shape (Table 2). The autotetraploid had fewer number of suckers, larger pseudostem, and had larger leaves compared to diploid (Table 2). The autotetraploid plants showed drooping leaves, while the control diploid banana exhibited upright leaves (Figure 2). The autotetraploid had more compact bunch and larger fruit diameter compared to the diploid (Figure 2). The autotetraploid had a truncated fruit apex, while the diploid had a bottle-necked fruit apex (Figure 2). These characteristics provide useful morphological traits for the screening of tetraploids. All tetraploids were maintained in the field for almost 3 years for evaluation of polyploidy stability; so far no major change has been seen on the morphological level.

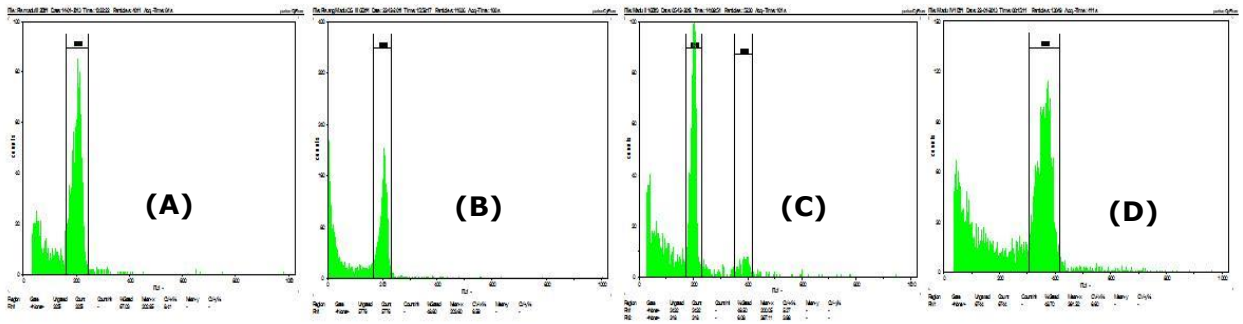

Figure 1. Histogram of: (A) Control banana (diploid), (B) Oryzalin-treated banana (diploid), (C) Oryzalin-treated banana (mixoploid), (D) Oryzalin-treated banana (autotetraploid).

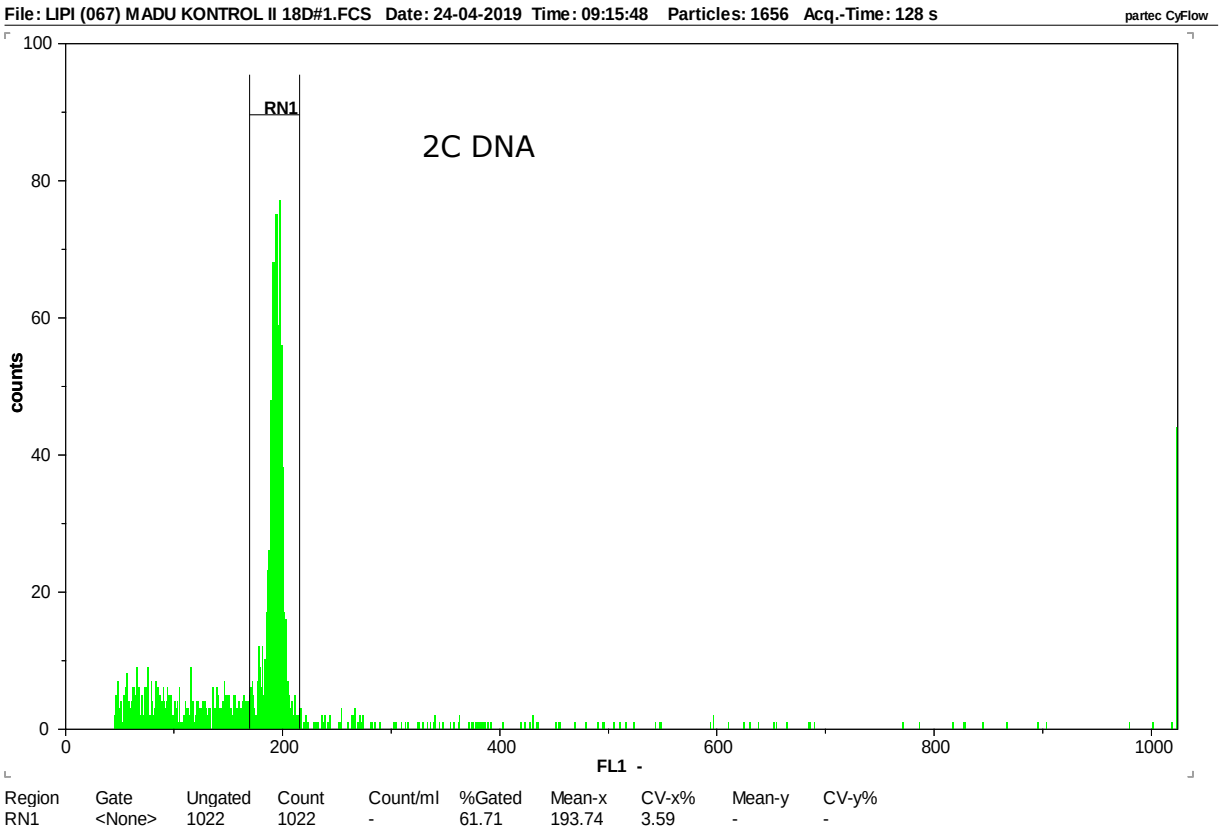

Figure 1.A. Histogram of Control "PisangMadu" (diploid).

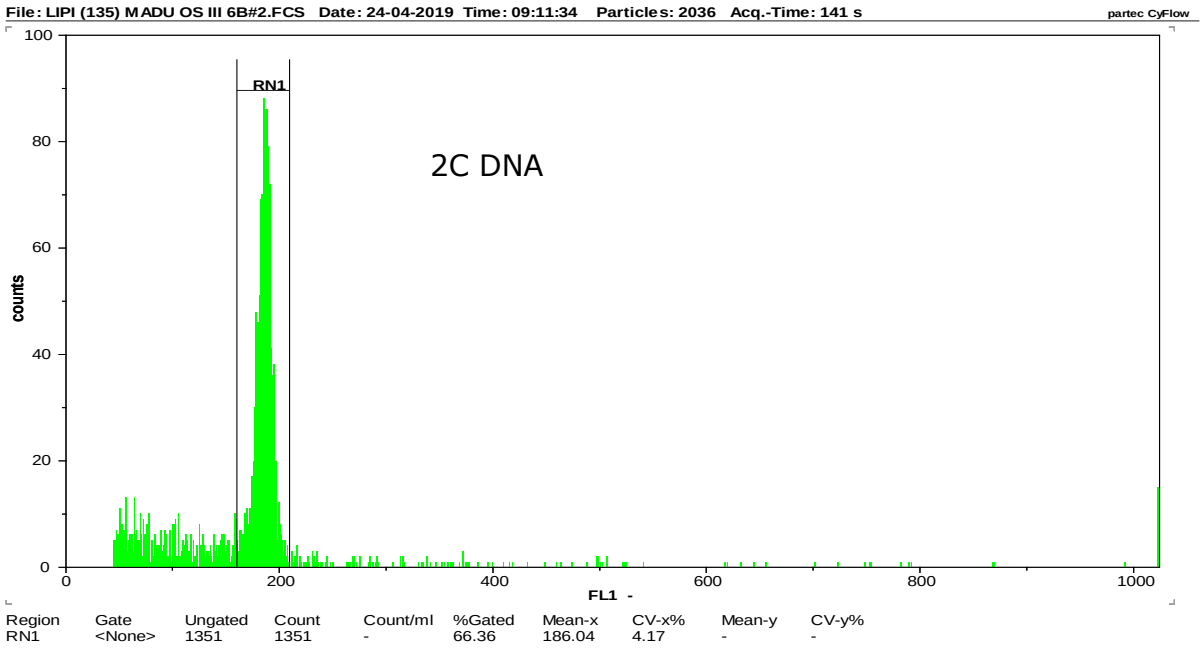

Figure 1.B. Histogram of Oryzalin-treated "PisangMadu" (diploid).

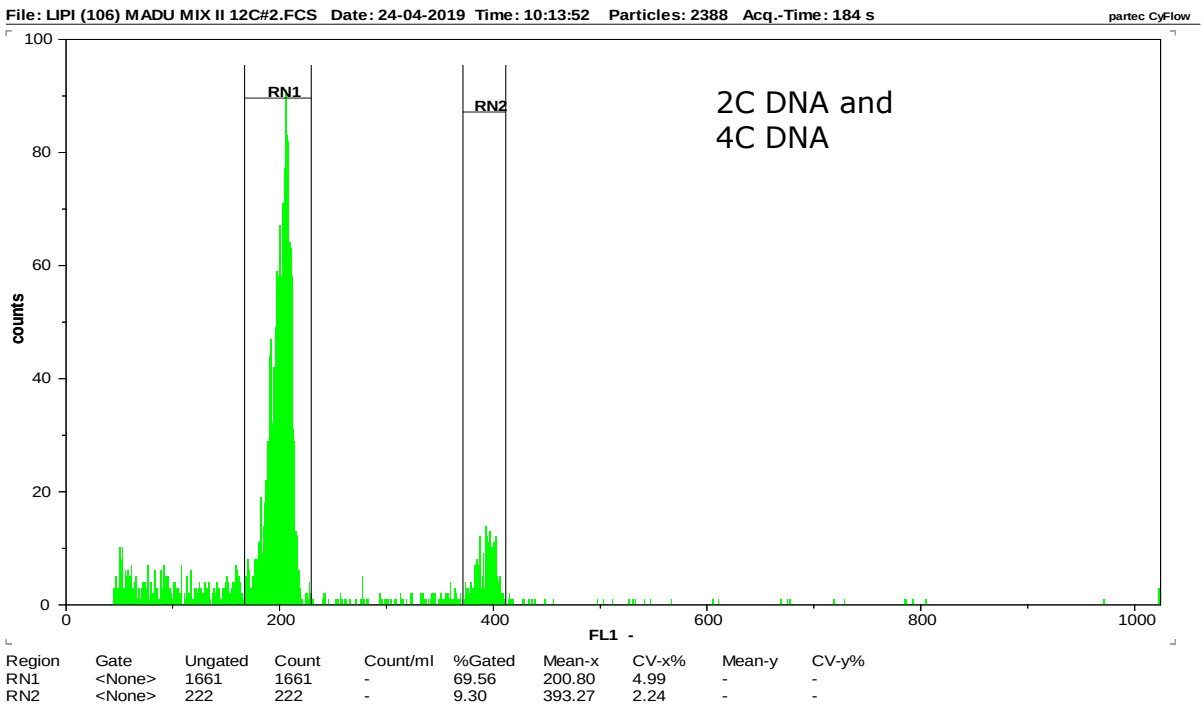

Figure 1.C. Histogram of Oryzalin-treated "PisangMadu" (mixoploid).

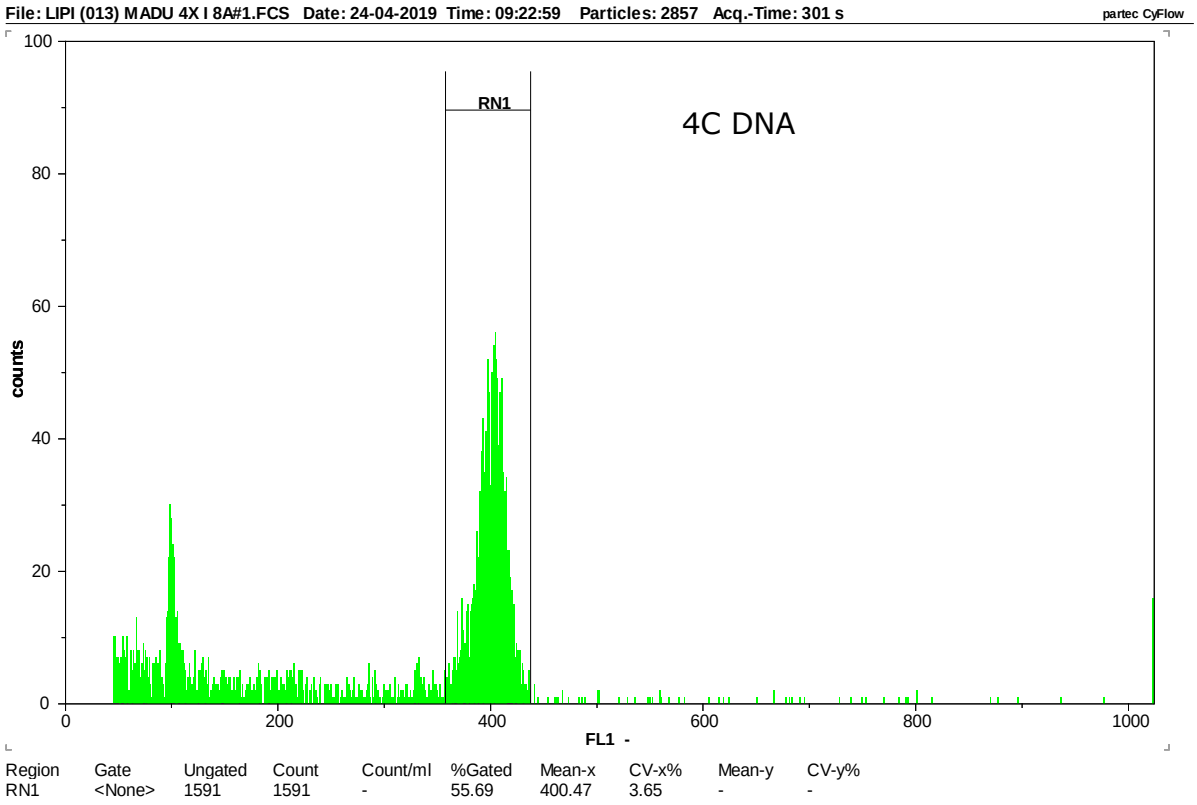

Figure 1.D. Histogram of Oryzalin-treated "PisangMadu" (tetraploid).

Table 2. Morphology characters of autotetraploid "Pisang Madu" compared to the diploid.

No.	Characters	Autotetraploid Pisang Madu	Diploid Pisang Madu
1	Ploidy	Tetraploid (AAAA)	Diploid (AA)
2	Rhizome: number suckers above ground	2.67±0.49	3.5±0.63
3	Pseudo stem: length (cm)	272.14±48.47	Short: : 227.25±20.52
4	Pseudo stem: diameter (cm)	17.25±1.79	13.73±1.81
5	Pseudo stem:overlapping of leaf sheaths	Medium	Medium
6	Pseudo stem:tapering	Medium	Medium
7	Pseudo stem:color	Light yellow green RHS 14A	Light yellow green RHS 2C
8	Pseudo stem:anthocyanin coloration	Medium	Medium
9	Pseudo stem:color of inner side of basal sheath	Light yellow green RHS 14A	Light yellow green RHS 2C
10	Plant: compactness of crown	Medium	Medium
11	Plant: growth habit	Drooping	Upright
12	Petiole:attitudewings at base	Curved outwards	Curved outwards
13	Petiole: length (cm)	36.86±5.9	36.62 ±8.33
14	Leaf blade: color of midrib on lower side	Yellow green RHS 145CD	Light yellow green RHS 2D
15	Leaf blade: shape of base	Both sides acute	Both sides acute
16	Leaf blade: waxiness on lower side	Weak	Weak
17	Leaf blade: length (cm)	187.71±25.62	167.12 ±25.24
18	Leaf blade: width (cm)	60.71±8.69	57.77±5.79
19	Leaf blade: ratio length/width	3.09	2.89
20	Leaf blade: glossiness at upper side	Present	Present
21	Peduncle: length (cm)	40.43±6.63	33.25 ± 5.28
22	Peduncle: diameter (cm)	5.05±0.33	4.54 ±0.65
23	Peduncle: pubescence	Present	Present
24	Peduncle: curvature	Medium	Medium
25	Bunch: length (cm)	45.86±2.67	46.62±9.05
26	Bunch: diameter (cm)	48.43 ±5.80	40.50±5.29
27	Bunch: shape	Irregular	Irregular
28	Bunch: attitude of fruits	Horizontal to slightly turned up	Horizontal to slightly turned up
29	Bunch: compactness	Compact	Medium
30	Bunch: number of hands	6.43 ± 0.79	7.62 ±2.39
31	Rachis: attitude of male parts	Vertical	Vertical
32	Rachis: prominence of scars	Strong	Strong
33	Rachis: persistence of bracts	Strong	Present
34	Rachis: persistence of hermaphrodite flowers	Present	Present
35	Fruit: curvature	Straight	Straight
36	Fruit: longitudinal ridges	Absent	Absent
37	Fruit: length (cm)	10.02±0.5	10.16 ± 0.35
38	Fruit: width (excluding ridges) (cm)	2.90 ± 0.11	2.57 ± 0.06
39	Fruit: length of pedicel (mm)	10.45±0.37	10.2 ± 0.6
40	Fruit: shape of apex	Truncated	Bottle-necked
41	Fruit: thickness of peel (mm)	2.82±0.50	Thin: 2.23 ±0.25
42	Fruit: color of peel before maturity	Dark green RHS 144A	Dark green RHS 144A
43	Fruit: color of peel	Dark orange yellow RHS 20B	Dark orange yellow RHS 20B
44	Fruit: adherence of peel	Medium	Medium
45	Fruit: persistence of floral organs	Present	Present
46	Fruit: color of flesh	Dark orange yellow RHS 16C	Dark orange yellow RHS 21B
47	Fruit: firmness of flesh	Firm	Firm
48	Male inflorescence: persistence	Present	Present
49	Male inflorescence: shape	Broad ovate	Medium ovate
50	Male inflorescence: opening of bracts	Closed	Closed
51	Bract: color ofinner side	Dark orange yellow RHS 16C	Dark orange red RHS 16C
52	Bract: shape of apex	Broad acute	Broad acute

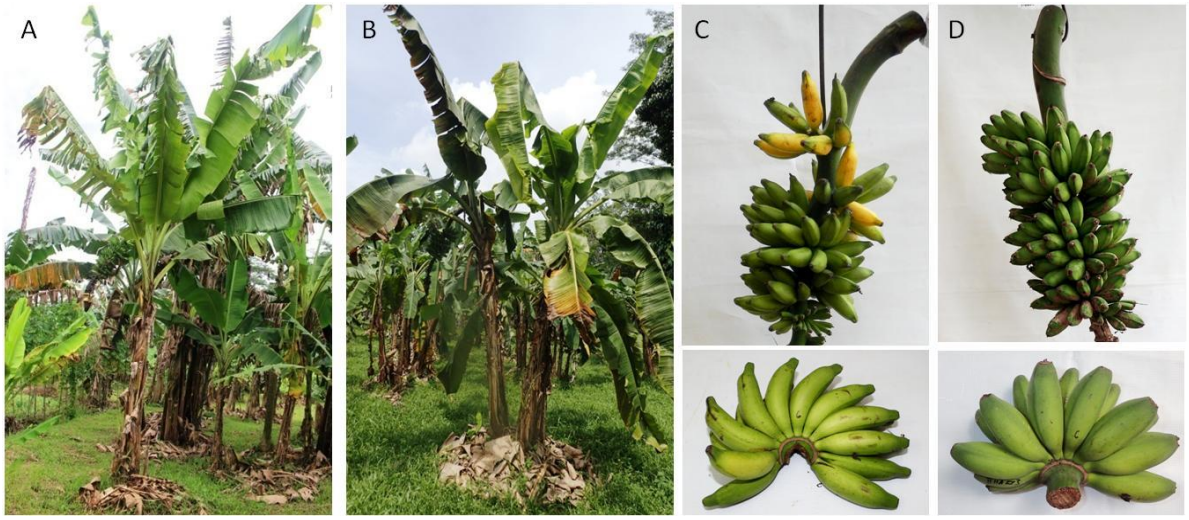

Figure 2. Characters of banana cultivar: (A) Plant habit of control diploid, (B) Plant habit of autotetraploid, (C) Bunch and Fruit bunch of control diploid (D) Bunch and fruit bunch of autotetraploid.

Table 3. Number of seed, embryo and ploidy levels of the hybrids.

Cross combination		No. of flowers pollinated	No of seeds	No of embryos	No of seedlings	Ploidy levels	
Female parent	Male parent					triploids	Diploids
Autotetraploid banana 4x	*Musa acuminata* var *malaccensis* 2x	87	42	23	12	12	0
Musa acuminata var *zebrina* 2x	Autotetraploid banana 4x	9	64	64	15	15	0

Secondary hybrid production

In order to evaluate the reproductive potential of the autotetraploid, hybridization with the wild varieties were carried out in this study (Table 3). When the autotetraploids were used as female parents (4x x 2x cross), 42 hybrid seeds were produced from 87 pollinated flowers. On the other hand, when the autotetraploid were used as male parents (pollen donor), a lot of hybrid seeds were obtained (84 hybrid seeds from 9 pollinated flowers). This result showed that the autotetraploid has good reproductive potential of female and male gametes, especially when crossed with wild varieties.

The hybrids were confirmed to be triploid (Table 4). The hybrids were normal and produced normal bunch and fruit (Figure 3).

Table 4. Ploidy levels of hybrids of MDMM and MZMD.

Hybrids	Ploidy	Mean	Sd
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	333.94	6.29
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	288.17	9.70
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	276.43	7.90
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	330.24	7.00
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	299.93	4.89
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	358.92	3.35
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	318.74	5.90
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	271.43	6.16
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	327.11	6.05
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	327.62	7.27
Autotetraploid banana 4x x *Musa acuminata* var *malaccensis* (MDMM)	3x	327.62	7.27
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	346.48	4.35
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	345.78	4.75
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	277.08	4.17
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	277.48	5.74
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	270.88	3.52
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	256.66	3.99
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	269.26	6.46
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	320.29	5.98
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	310.43	4.81
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	340.14	5.62
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	309.70	3.23
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	295.64	2.95
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	284.23	4.51
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	288.85	3.70
Musa acuminata var *zebrina* xautotetraploid banana 4x (MZMD)	3x	303.02	4.64

Figure 3. Performance of hybrid bunches of MDMM and MZMD.

DISCUSSION

Ploidy identification

Van Duren *et al*. (1996) used flow cytometry to identify autotetraploid banana plants induced through chromosomal doubling of diploid material. The phenomenon of mixoploidy was also commonly encountered in this work, and flow cytometry was efficient at detecting these cases (Pio *et al*., 2014). Mixoploids may arise because antimitotic agents may not always reach all of the meristems on a plant (or those that are actively dividing) (Carvalho *et al*., 2005).

Tetraploid identification could be conducted by chromosome counting (Vakili, 1967, Hamill, 1992, Osuji *et al*., 1996*).* However, this technique was quite difficult because the chromosome size of banana is very small and time consuming. Therefore, the use of chromosome counting was limited to screen induced polyploidy (Asif *et al.,* 2000). As the effect of genotype and mixoploid was difficult to identify, chromosome

counting was not recommended for screening of induced polyploidy (van Duren *et al.,* 1996).

Another technique to estimate ploidy level was stomata measurement in terms of the amount, size, and density of stomata (Blanke *et al.,* 1994). The technique was easier compared to chromosome counting; however, it might not be reliable since there was environmental effect (van Duren *et al.,* 1996). Therefore, flowcytometry technique was preferred because it's faster, precise, and simple for ploidy screening (Asif *et al*., 2000).

In this study, 57.14% of treated plants were autotetraploids, 11.9% were mixoploid, and 30.96% were diploids (Table 2). The mixoploid plants had fewer suckers, drooping leaves, bigger size of pseudo stem, and bigger fruit compared to their diploids. Regenerated plants obtained from oryzalin treatments showed slower growth and thicker leaves compared to diploid controls.

The induction of chromosome doubling depends on a large number of variables: media, antimitotic agents, explants types, exposure times, and concentrations. Flow cytometry is the pre-eminent method for evaluation of the induced polyploidization. Alternative confirmation methods, such as chromosome counts and morphological observations are also used. However, in the study by Bakry *et al.* (2007) that was conducted on a wide range of mono- and interspecific diploid banana clones, it was observed that chromosome counts led to distinguish the diploid from tetraploid plants but did not detect chimeras, while flow cytometry allowed an early screening of a larger number of plants that rapidly detects chimerical plants.

Morphological characteristics of autotetraploid banana

Tetraploid banana plants showed larger pseudostem, more robust, had drooping leaves, longer growth period, fewer number of suckers, and had less root system compared to the diploid (Vakili, 1967). Tetraploidy affected fruit size and shape of *Musa balbisiana*and*Musa acuminata* subsp. *banksii.* Tetraploidy did not affect bunch size of *Musa acuminata* subsp. *banksii*, but reduced bunch size of *Musa acuminata* subsp. *microcarpa 'zebrina'* (Vakili, 1967). Hamill *et al.,* (1992) stated that autotetraploids had bigger leaves, fragile petiole, drooping leaves, bigger diameter of pseudostem, and fewer suckers compared to the original diploid. Autotetraploid plants had longer, broader leaves, and bigger bunch size compared to diploid *Musa acuminata*' KluaiLeb Mu Nang' and 'Kluai Sa' (Kanchanapoom and Koarapachaikul, 2012). Colchicine-induced tetraploids from 21 diploid clones showed weak tetraploid plants compared to their diploids. However, all tetraploids produced flowers and could be crossed with diploids to generate triploid hybrids (Bakry *et al*., 2007).

In this study, the autotetraploid "Pisang Madu" plants exhibited drooping leaves, larger pseudostem compared to the diploid, as observed by Vakili (1967) and Hamill *et al*. (1992). The autotetraploids had larger fruit size and bunch compared to the diploid, as observed by Amaral *et al*. (2015) and Kanchanapoom and Koarapachaikul (2012). The auto tetraploid "Pisang Madu" had drooping leaves, while diploid "Pisang Madu" had upright leaves. Similar results were also observed in "Pisang Lilin"

and "SH-3362 (Amaral *et al*., 2015; Hamill *et al*., 1992). These characteristics provided useful morphological traits for the screening of tetraploids. The autotetraploid "Pisang Madu" produced flowers and could be crossed with diploids to generate triploid hybrids, as observed by Bakry *et al*. (2007). All tetraploids were maintained on the field for almost three years for evaluation of polyploidy stability; so far no major change has been seen on the morphological level.

CONCLUSION

Inducing autotetraploidy in banana have a significant impact on breeding program as this will reduce the time needed, manpower expenditures, and costs involved in obtaining tetraploids as compared to conventional methods. The autotetraploids obtained in this study may not be necessarily be recommended as cultivars, as tetraploids generally have drooping and fragile leaves, so it will be necessary to obtain triploid plants from them. The autotetraploid plants were successfully crossed with diploid varieties in the germplasm of RCB IIS to generate triploid hybrids.

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