

# Genome group classification and diversity analysis of talas and rutai banana, two local cultivars from East Kalimantan, based on morphological characters

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**Abstract.** Sunaryo W, Mulyadi A, Nurhasanah. 2019. Genome group classification and diversity analysis of talas and rutai banana, two local cultivars from East Kalimantan, based on morphological characters. *Biodiversitas* 20: 2355-2367. Talas and rutai banana are two local cultivars grown in East Kalimantan and long-time used as dessert or cooking bananas for traditional food and cakes, but unfortunately, their taxonomic and genetic status are still little known. This study was conducted to explore their taxonomic and genetic status through morphological observation, genomic group classification and diversity analysis of talas and rutai banana compared to other recognized and identified bananas in Indonesia, i.e., liar/monyet, Ambon, tanduk and klutuk banana. In total 108 morphological characters were observed on site of growing area using the “*Descriptors for Banana (Musa spp.)*” morphological indicators. The genome group classification was carried out using Simmonds and Shepherd scoring system toward fifteen key banana characters. The diversity analysis was performed using Hierarchy Cluster Analysis at MINITAB 17.1 software based on the selected characters of the highest Principal Component Analysis (PCA) values. The morphological observation showed that the characters of talas and rutai banana are much closely related to those of belonging to *M. acuminata* although some characters showed *balbisiana*-type. In addition, liar/monyet and klutuk banana morphological characters were exactly matched with the *M. acuminata* and *M. balbisiana* accessions. Furthermore, most of Ambon banana morphological characters are similar to *M. acuminata*, but tanduk banana had a combination characters between *M. acuminata* and *M. balbisiana*. The genome group classification showed that talas and rutai banana are the AAB genotype, while liar/monyet, Ambon, tanduk, and klutuk were grouped into AA, AAA, AB, and BB genotype, respectively. The diversity analysis showed that talas and rutai banana have a very high similarity about 75% and closed related to AA/AAA genotype i.e., liar/monyet and Ambon with similarities level of 45%.

**Keywords:** Talas and rutai banana, morphological observation, genome classification, diversity analysis, East Kalimantan

## INTRODUCTION

Bananas (*Musa paradisiaca*), monocotyledons herbs, have played an important role in food supply mainly in tropical and subtropical countries served as dessert or cooking bananas (Lescot 2011). Banana is very good for human diet due to the high nutritional contents such as carbohydrates, vitamins, and minerals (Wall 2006). Furthermore, bananas are affordable fruit and abundantly available in the local or supermarket since they can be cultivated in very broad range of climate and environment condition.

There are around 1940 cultivars of recognized bananas in the world (Crichton et al. 2016). The hybridization and genetic mutation of the diverse species and subspecies have enlarged the genetic diversity of banana cultivars and landraces (Heslop-Harrison and Schwarzacher 2007). The edible bananas are selected for seedless, parthenocarp-developed fruit, triploidy genome (Simmonds and Shepherd 1955; Simmonds 1962; Valmayor et al. 2000). The domestication of banana is considered by the economic value of the banana cultivars for food and industries. The modern bananas, referring to the bananas cultivated in the largest area of cultivation for food and industries, are

usually derived from the triploid seedless banana as a result of hybridization and genetic improvement of banana ancestor i.e. *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Espino et al. 1992). Those are classified as *Eumusa* characterized by the chromosome number of  $2n = 2x = 22$  (Wong et al. 2002). For the triploid seedless bananas, the fruit parthenocarpic development has occurred since there is a failure of fertilization (Horry et al. 1997).

The formation of modern bananas involved their ancestors i.e. *M. acuminata* referred to A-genome, and *M. balbisiana* referred to B-genome. A-genome is characterized by the sweet and dessert banana, whereas, the B-genome is referred for the hardness and starchiness banana (Pillay et al. 2002). There are specific different morphological characters between both species. Simmonds and Shepherds (1955) used these characters to classify the genome group of their interspecific triploid hybrids such as triploid hybrids of plantains (AAB genotype) and other cooking bananas (ABB genotype). Furthermore, the interspecific hybrids of both species can be referred to as *Musa x paradisiaca* (Espino et al. 1992).

Indonesia is one of the diversity centers of *M. acuminata* and *M. balbisiana* species (Purseglove 1985;

Horry et al. 1997) and there are more than 325 cultivars/clones which 14 of them are domesticated and cultivated for food and small industries by Indonesian people (Setyadjit et al. 2003). The contribution of bananas to Indonesian economy cannot be neglected since bananas is the most important fruit commodity with the highest fruit production of 7,128,698.6 ton in 2017 (Directorate General of Horticulture, Ministry of Agriculture, Republic of Indonesia 2017). Therefore, many efforts have been donated to explore, identify and conserve Indonesian banana germplasms. Many clones commercially cultivated by farmer are still unidentified and recognized including those are existing in Borneo/Kalimantan Island.

Talas and rutai banana, two local cultivar bananas from East Kalimantan, have been cultivated for long years ago, but the taxonomic and genetic status are still unknown. The morphological observation and characterization of talas banana have been reported (Sunaryo et al. 2017), but the same observation has not been performed for the rutai banana. Talas banana is a superior variety showing high agronomic performances, nutritional status and economic value such as high yield and fruit performance at an appropriate cultivation input, good taste, soft texture, sweet, not astringent or sour, high starch content, low water content, and long shelf-life. The fruit is unique and interesting, yellow-orange pulp color and long-curved fruit apex. The high economic value is indicated by the high price in local market (IDR 20,000-40,000 per hand) compared to other commercial bananas (Sunaryo et al. 2017). Based on the initial observation, rutai banana does not possess good agronomic performance since the fruit is tiny, therefore the yield per hectare is very low about 3-5 kg per bunch. However, the fruit of rutai banana has a unique shape with lengthily pointed apex and curved fruit shape. The nutritional value and the fruit performance have not been studied, but the fruit is very delicious, easily peeled, very sweet and very soft therefore it is preferred by children and old people. The price in the local market is also very high showing good economic value of this cultivar.

Banana genome classification using morphological characters has been widely known (Simmonds and Shepherd 1955; Silayoi and Chomchalow 1987; Pillay et al. 2004). They used 15 remarkable morphological and distinctive characters for taxonomy scoring in genome classification as proposed by Simmonds and Shepherds (1955). This taxonomy scoring method is still reliable and feasible for *Musa* spp., but it has limitation in the genome determination especially for *M. Schizocarpa* and *Australimusa* species (Phillay et al. 2004). The genome classification and diversity analysis of Indonesian bananas were reported (Damayanti 2007; Sukartini 2007; Wahyuningsih et al. 2009; Retnoningsih 2009; Rinaldi et al. 2014; Fitriyah et al. 2017). In the previous genetic diversity studies, talas and rutai banana were excluded since they were little known and limited grown. This article reported the genome group classification and diversity analysis of talas and RUTAI banana, two local cultivars from East Kalimantan, based on morphological characters.

## MATERIALS AND METHODS

### Plant materials

Two local cultivars from East Kalimantan (talas and rutai bananas), and four other cultivars i.e. Ambon (Genome AAA), tanduk (ABB), liar/monyet (AA) and klutuk (BB) were used in this research. Liar/monyet and *Kluthuk* bananas was used in this study since they are known and identified as diploid *Musa acuminata* and *Musa balbisiana* cultivars and the ancestor of modern bananas (Simmonds and Sheperd 1955). These cultivars were used as genome group control for the banana target identification according to Simmonds and Shepherd genome classification system. The Ambon and tanduk bananas were used in this study to represent the cultivar possessing A and B genome. All banana trees from the 6 cultivars were observed on-site by field visit to the determined grown area (local farmer field) located at Kutai Kertanegara, Paser and Samarinda districts, East Kalimantan Province, Indonesia.

### Plant morphological observation

The morphological characters were observed for plant identification based on the criteria of plant and fruit morphological characters in the "*Descriptors for Banana (Musa spp.)*" (INIBAB 1996). The flower morphology was specifically analyzed to observe the morphology of male and female flower organ determining the normal or abnormality of the flower and the male or female sterility. The normal or abnormality of banana flower will show the fruit seed identity and possibly will also represent the ploidy of the banana genome. The mature banana flowers were collected from the fresh banana male buds. The bracts of male bud were opened and sectioned, thereafter, separated from the male bud to obtain the fresh mature intact flowers. The whole plant and specific organ pictures such as leaves, pseudostem, male bud, suckers, bunch, flower and fruit were taken using a Nikon Camera.

### Genome group classification

The classical genome group classification was employed as described by Simmonds and shepherd (1955) and updated by Stover and Simmonds (1987). Besides the Simmonds and Shepherd scoring system, the other system as described by Silayoi and Chamchalow (1987), Singh et al. (2014), and Rinaldi et al. (2014) were also used. The score of fifteen banana key characters was recorded and inputted in the scoring tables. The fifteen characters as described by Simmonds and Shepherd system are the key characters of two ancestors of modern banana i.e. *M. acuminata* and *M. balbisiana* (Table 1). Each banana cultivar will be scored as 1, 2, 3, 4 and 5, based on their morphological characters. The score 1 is for the cultivar similar to/or has high similarity to *M. acuminata* whereas score 5 is for those similar to/or has high similarity to *M. balbisiana*. Score 2-4 is the intermediate score determined by identifying the similarity level of the samples to *M. acuminata* or *M. balbisiana*. The total score of each banana variety, thereafter, was compared to the genome score indication as described in Table 2.

**Table 1.** Fifteen key characters of *Musa acuminata* and *Musa balbisiana* according to Simmonds and Shepherd's (1985)

No	Character	<i>Musa acuminata</i>	<i>Musa balbisiana</i>
1	Pseudostem color	More or less heavily marked with brown or black blotches	Blotches very slight or absent
2	Petiole canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin inclosed, not winged but clasping pseudostem
3	Peduncle	Usually downy or hairy	Glabrous
4	Pedicels	Short	Long
5	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6	Bract shoulder	Usually high (ratio<0.28)	Usually low (ratio>0.30)
7	Bract curling	Bracts reflex and rollback after opening	Bracts do not reflex
8	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9	Bract apex	Acute	Obtuse
10	Bract color	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11	Color fading	Inside bract color usually fades to yellow towards the base	Inside bract color usually continuous to base
12	Bract scars	Prominent	Scarcely prominent
13	Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14	Male flower color	Creamy white	Variably flushed with pink
15	Stigma color	Orange or rich yellow	Cream, pale yellow or pale pink

**Table 2.** Determinant scoring of genome group classification based on the morphological characters in banana (Table was adopted from Singh et al. (2014) and Rinaldi et al. (2014))

Genome group	Simmonds and Shepherd (1982)	Silayoi and Chamchalow (1987)	Singh et al. (2014)	Rinaldi et al. (2014)
AA/AAA	15-23	15-27	15-25	15-25
AAB	24-46	26-46	26-45	26-46
AB	49	-	46-49	47-58
ABB	59-63	59-63	59-65	59-63
ABBB	67	-	66-69	64-75
BB/BBB	-	70-75	70-75	-

### Data analysis

The plant diversity was analyzed using 108 morphological characters adopted from the banana descriptor (INIBAB 1996). The diversity analysis was performed using the Hierarchy Cluster analysis at Average Linkage (*between groups*) at MINITAB 17.1 software to obtain the similarity level and the genetic relationships among varieties showing by a dendrogram. The dendrogram will show the genetic relationships and distance among groups and accessions. The raw data for the diversity analysis were obtained from the conversion of morphological characters by *Principal Component Analysis* (PCA) and thereafter they were selected for only the characters producing the PCA values more than 0.750. The higher PCA value the higher level of characters to discriminate the accessions differences.

## RESULTS AND DISCUSSION

### Plant morphology

Liar/monyet and klutuk banana were used in this study suggested as representatives of *M. acuminata* and *M.*

*balbisiana* respectively, the ancestors of modern banana (Simmonds and Sheperd 1955; D'Hont et al. 2012), as control of banana identification and characterization. Liar/monyet banana has been identified as a *M. acuminata* species (Retnoningsih 2009; 2014) with AA genome (sweet banana), whereas klutuk is characterized as *M. balbisiana* cultivar with BB genome or referred to cooking banana (Sumardi and Wulandari 2010; Retnoningsih et al. 2014; Fitriyah et al. 2017).

The morphological observation of liar/monyet banana resulted in the unsurprisingly similar indication as a *M. acuminata* showing remarkably characters such as dark brown blotches in pseudostem, U-shaped canal petiole, very hairy peduncle, very short pedicels, the reflexed bracts after opening, lanceolate bract shape, acute bract apex, very prominent bract scars, two irregular rows of ovules in each loculus, horizontal rachis position, and red fading to yellow internal bract color (Table 3, Figure 1.A). In addition, liar/monyet banana is an inedible banana, because it contains very dense seeds in fruit aril indicating a diploid AA genome of *M. acuminata* species. These results indicate that liar/monyet banana can be used as representative of *M. acuminata* in this study since it shows a true *M. acuminata* characters.

The observation of klutuk banana showed remarkably *M. balbisiana* properties as reported by Simmonds and Sheperd (1955) such as very few blotches on the pseudostem, inward margin of the petiole canal, glabrous peduncle, long pedicels, four irregular rows of ovules, un-reflexed bracts before falling, broadly ovate bract shape, obtuse bract apex, scarcely prominent bract scars, bright crimson color in internal bract, and vertically rachis position (Table 3, Figure 1.B). The seeds composing of klutuk banana fruit indicates that this cultivar is a diploid BB genome of *M. balbisiana*. These indicate that klutuk banana used in this study is a true *M. balbisiana* and can be

used as representative of this accession. Other report showed that there is another “*klutuk*” called as “*klutuk susu*” but it had AAB genome with some character differences compared to *M. balbisiana* characters (Fitriyah et al. 2017).

Morphological observation of talas and rutai banana showed a combined character derived from the banana ancestors i.e. *Musa acuminata* (Genome AA) and *Musa balbisiana* (Genome BB). As reported by Simmonds and Shepherd (1955), both banana ancestors have inherited many modern bananas, including talas and rutai banana, via natural or artificial hybridizations, genetic mutation and genetic improvement (Espino et al. 1992; Dolezel et al. 1994). The morphological characterization showed that talas banana has remarkable characters similar to those of belonging to *M. acuminata* such as brown or black blotches in pseudostem, U-shaped margin canal petiole (straight with erect margins), hairy peduncle, the reflexed bracts before falling, lanceolate/ovate bract shape, pointed bract apex, prominent bract scars, and creamy white of male flower color (Table 3, Figure 1.C.). Whereas, some characters are dominantly derived from the *M. balbisiana* ancestor such as slightly angled hanging bunch, short peduncle with the length less than 30 cm, vertically falling rachis, medium crimson bright color at internal bract, curved fruit shape with lengthily pointed long fruit apex like a plantain, long pedicle, unfused pedicels that can be separated easily, difficult peeled-fruit, persistent fruit at the pedicle, and vertically rachis and bunch position (Table 3, Figure 1.C). Other specific interesting properties are that talas banana is classified as a medium-size banana (2.1-2.9m) with a slimy pseudostem, and medium plant height (pseudostem height) between 2-3 m (not giant or dwarf banana) (Table 3, Figure 1.C). Most cultivated bananas have pseudostem height in the range of 2-9 m (INIBAB 2000). The bunch is hanging slightly angled on the pseudostem and classified as a medium-size bunch. These results confirm the previous morphological observation as reported by Sunaryo et al (2017). Furthermore, Sunaryo et al (2017) reported that the mature fruit of talas banana showed seedless, soft texture, sweet and tasty properties with medium fruit aril hardness, therefore, it can be used as dessert or cooking banana. These properties are supported by the high content of starch (carbohydrates) in the fruit aril.

Rutai banana is an endemic local banana originated from East Kalimantan classified as dwarf or tiny edible banana with very short pseudostem height (< 2 m) and very slimy pseudostem diameter (Table 3, Figure 1.D). The mature fruit is sweet, soft, tasty, and delicious with the size as big as human fingers, therefore the bunch weight was very low (< 5 kg). The pseudostem of rutai banana is scarcely blotched with red-purple indicates rutai banana has intermediate pseudostem character between *M. acuminata* and *M. balbisiana*. Among banana characters, the morphology of pseudostem is broadly varied both in length and color (Pillay and Tripathi 2007). Furthermore, the key characters such as petiole canal shape, peduncle surface, bract color and scars, bract shape and apex, ovule arrangement, bract curling, horizontal rachis, and bunch

position and flower and stigma color were similar to the *M. acuminata* characters (Table 3, Figure 1.D). The other interesting properties are long fruit pedicels, lengthily pointed fruit apex, curved fruit, easily peeled fruit, and easily fruit fall from hands (*deciduous*). The mature fruit appeared very bright yellow. This observation indicates that some morphological characters of rutai banana belong to *M. balbisiana*. It can be concluded that the morphological characters of rutai banana are intermediate characters between *M. acuminata* and *M. balbisiana*, but generally, it is closer to *M. acuminata*.

Ambon and tanduk banana are broadly and well known among Indonesian commercial bananas. Ambon is the representative of dessert banana and identified as AAA genome banana (Retnoningsih 2009; Retnoningsih et al. 2014; Rinaldi et al. 2014). On the other hand, tanduk banana is a plantain or cooking banana that has been broadly used as foods and cakes. It is reported as AAB genome (Retnoningsih et al. 2014). The morphological observation showed that Ambon banana is a medium-size banana with pseudostem height ranged from 3.0 to 4.0m (Table 3, Figure 1.E). The pseudostem is green yellowish colored with dark-brown blotches. Most key characters of Ambon banana observed showed a very close of those belonged to *M. acuminata* characters (Table 1, Figure 1.E) such as the U-shaped petiole canal, dark brown blotches, reflexed bracts before falling, two rows ovule arrangement, very short pedicels, stopped fruit apex, very prominent bract scars, lanceolate bract shape, pointed/acute bract apex, purple to yellow bract color, and yellow male flower and stigma (Table 3, Figure 1.E). The other prominent character of Ambon banana is the bunch hanging vertically composed of dense fruit and hand. The fruit is soft, easily peeled, and seedless. The mature fruit peel is green (or green yellowish depending on the variety). Ambon banana is very commonly used as dessert/sweet banana.

tanduk banana is well known due to the giant/big fruit size (25-35 cm fruit length and 6-8 cm fruit diameter). In other Indonesian areas, it is called as *Agung* or *Byar* banana. The prominent morphological characters of tanduk banana, beside the size, are the outward curved fruit shape that is uncommon/abnormal for banana character (Table 3, Figure 1.F). The other interesting properties are the low number of hands composed about 2-5 hands of the bunch. The fruit has long shelf life; therefore it is appropriate as cooking banana. As a plantain, tanduk banana shows the hard texture of fruit aril even in mature fruit indicating high content of carbohydrates. The pseudostem of tanduk banana is slimy medium size with dark green yellowish color containing very few blotches, which is an intermediate character of pseudostem color between *M. acuminata* and *M. balbisiana*. The peduncle is glabrous and the bract scars are not prominent (Table 3, Figure 1.F). The fruit is seedless and emerging from the lanceolate male bud. The male bud is composed of fractured lanceolate bracts. The bracts are very slightly reflexed. These indicate that there are some tanduk characters derived from *M. balbisiana*.

**Table 3.** Morphological characters of talas and rutai banana compared to other four local cultivars from East Kalimantan based on the *Banana Descriptor* (INIBAB 1996)

Character	Banana cultivar					
	Talas/sunking	Rutai	Tanduk	Ambon	Liar/monyet	Klutuk
Leaf habit	Intermediate	Erect	Intermediate	Intermediate	Erect	Intermediate
Dwarfism	Normal	dwarf	Normal	Normal	Normal	Normal
Pseudostem height (m)	2.1-2.9	1.5-2.5	2.5-3.4	3.0-4.0	>3	>3
Pseudostem aspect	Slender	Slender	Slender	Normal	Slender	Robust
Pseudostem color	Green dark brown	Red-purple	Green-yellow	Green dark-brown	Green dark brown	Dark Green
Pseudostem appearance	Shiny	Shiny	Dull	Dull	Shiny	Dull
Pseudostem predominant color	Green	Pink-purple	Light green	Light green	Watery green	Green
Pigmentation underlying pseudostem	Red	Pink-purple	Purple	Yellow-Purple	Pink-purple	Green-purple
Sap color	Watery	Watery	Watery	Milky	Watery	Milky
Wax on leaf sheaths	Moderately waxy	Moderately waxy	Very few wax	Moderately waxy	Very little	Very waxy
Development of suckers	Between ¼ and ¾ of the height of the parent plan	Between ¼ and ¾ of the height of the parent plan	Between ¼ and ¾ of the height of the parent plant	More than ¾ of the height of the parent plant	More than ¾ of the height of the parent plant	Between ¼ and ¾ of the height of the parent plan
Sucker position	Close the parent (vertical growt)	Close the parent (vertical growt)	Close the parent (vertical growt)	Close the parent (vertical growt)	Close the parent (vertical growt)	Close the parent (vertical growt)
Blotches at the petiole base	Small blotching	Small blotching	Sparse blotching	Sparse blotching	Large Blotches	Sparse blotching
Blotches color	Black purple	Brown-black	Dark Brown	Brown-black	Brown-black	Green-black
Petiole canal leaf III	Straight with erect margins	Straight with erect margins	Margins curved inward	Straight with erect margins	Straight with erect margins	Margins curved inward
Petiole margins	Winged and not clasping the pseudostem	Winged and not clasping the pseudostem	Winged and undulating	Winged and not clasping the pseudostem	Winged and not clasping the pseudostem	Not winged and clasping the pseudostem
Wing type	Not dry	Dry	Dry	Dry	Not dry	Dry
Petiole margin color	Pink-purple to red	Pink-purple to red	Pink-purple to red	Pink-purple to red	Pink-purple to red	Green
Edge of petiole margin	With a color line along	With a color line along	With a color line along	With a color line along	With a color line along	Colorless
Petiole margin width	≤ 1 cm	≤ 1 cm	> 1cm	≤ 1 cm	> 1cm	≤ 1 cm
Leaf blade length (cm)	221-260 cm	≤ 170 cm	≤ 170 cm	171-220 cm	221-260 cm	>261 cm
Leaf blade width (cm)	≤ 70 cm	≤ 70 cm	≤ 70 cm	71 s.d 80 cm	≤ 70 cm	81 s.d 90 cm
Petiole length	51-70 cm	≤ 50 cm	51-70 cm	≤ 50 cm	51-70 cm	≤ 50 cm
Color of leaf upper surface	Green	Dark green with red purple	Dark green with red purple	Dark green with red purple	Dark green with red purple	Dark green
Appearance of leaf upper surface	Dull	Dull	Shiny	Dull	Shiny	Dull
Color of leaf lower surface	Green yellow	Green	Green	Medium green	Green	Dark green
Appearance of leaf lower surface	Dull	Dull	Shiny	Dull	Shiny	Dull
Wax on leaves	Few waxy	Moderately wax	Moderately wax	Few waxy	Few waxy	Very waxy
Insertion point of leaf blades on petiole	Asymetric	Asymetric	Asymetric	Symetric	Asymetric	Simetric
Shape of leaf blade base	Both side pointed	Both side pointed	Both side rounded	One side rounded, one pointed	One side rounded, one pointed	Both side rounded
Leaf corrugation	Even, smooth	Few stripes	Very corrugated	Few stripes	Even, smooth	Very corrugated
Color of midrib dorsal surface	Light green	Pink-purple	Pink-purple	Light green	Light green	Green
Color of midrib ventral surface	Light green	Pink-purple	Pink-purple	Light green	Light green	Green

Color of cigar leaf dorsal surface	Green	Red-purple	Red-purple	Green	Red-purple	Green
Blotches on leaves of water sucker	Little or narrow blotches	Little or narrow blotches	Little or narrow blotches	Little or narrow blotches	Large purple blotches	Without blotches
Peduncle length (cm)	≥ 21 cm (long)	11-20 cm (medium long)	≥ 21 cm (long)	≤ 10 cm (short)	≤ 10 cm (short)	≥ 21 cm (long)
Peduncle width (cm)	≤ 6 cm	6-12 cm	>13 cm	>13 cm	6-12 cm	>13 cm
Peduncle color	Dark green	Dark green	Dark green	Dark green	Dark green	Green
Peduncle hairiness	Slightly hairy	Slightly hairy	Hairless	Slightly hairy	Very hairy	Hairless
Bunch position	Slightly angle	Horizontal	Hanging Vertically	Slightly angle	Slightly angle	Horizontal
Bunch shape	Spiral	Spiral	Truncated cone shape	cylindrical	Spiral	Asymetric
Bunch appearance	Compact	Compact	Lax	Compact	Compact	Very compact
Flowers that form fruits	Female (absence of pollen sacs or pollens)	Female (absence off pollen sacs or pollens)	Female (absence of pollen sacs or pollens)	Female (absence of pollen sacs or pollens)	Hermaphrodite (presence of pollen sacs or pollens)	Hermaphrodite (presence of pollen sacs or pollens)
Male flower color	Cream	Yellow	Cream	Yellow	Cream	Yellow
Positioning of fruits on the crown	Uniseriate	Biseriate dan fused	Biseriate	Biseriate	Biseriate	Uniseriate
Rachis type	Present and male bud may be degenerated or persistent	Present and male bud may be degenerated or persistent	Truncated, no bract scar below the last hand of fruit	Present and male bud may be degenerated or persistent	Present and male bud may be degenerated or persistent	Present and male bud may be degenerated or persistent
Rachis position	Falling vertically	Horizontal	At an angle	With a curved	Horizontal	Falling vertically
Male bud type	Present	Present	Absent	Present	Present	Present
Male bud shape	Intermediate	Like a top	Fractured	Intermediate	Like a top	Ovoid
Male bud size	21-30cm	≤ 20 cm	≥ 31 cm	≥ 31 cm	≤ 20 cm	21-30cm
Bract base shape/shoulder	Medium	High	High	High	High	Low
Bract apex shape	Medium acute	Acute	Acute	Acute	Acute	Obtuse
Bract imbrication	Young bractea greatly overlap	Young bractea slightly overlap	Young bractea greatly overlap	Old bractea overlap at apex of bud	Old bractea overlap at apex of bud	Young bractea slightly overlap
Color of the bract external face	Dark purple	Dark purple-yellow	Dark purple	Dark purple-yellow	Dark purple-yellow	Dark purple-bright crimson
Color of the bract internal face	Bright crimson-yellow	Purple-yellow	Bright crimson-yellow	Purple-yellow	Cream-yellow	bright crimson
Color on the bract apex	Discolored	Discolored	Discolored	Discolored	Discolored	Color is uniform until apex
Color stripes on bract	With Discolored lines	Without discolored lines	With Discolored lines	With Discolored lines	With Discolored lines	Without discolored lines
Bract scars on rachis	Very prominent	Very prominent	Not prominent	Very prominent	Very prominent	Not prominent
Fading of color on bract base	Red fades to yellow	Red fades to yellow	Red fades to yellow	Red fades to yellow	Red fades to yellow	Continuosly red
Male bract shape	0.28 < x/y < 0,30 (medium ovate)	x/y < 0.28 (narrowly ovate/lanceolate)	x/y < 0.28 (narrowly ovate/lanceolate)	x/y < 0.28 (narrowly ovate/lanceolate)	x/y < 0.28 (narrowly ovate/lanceolate)	x/y > 0.30 (broadly ovate ovate)
Male bract lifting	Lifting two or more at a time	Lifting one time at a time	Lifting one time at a time	Lifting one time at a time	Lifting one time at a time	Lifting two or more at a time
Bract behaviour before falling	Bracts reflex	Bracts reflex	Bracts do not reflex	Bracts reflex	Bracts reflex	Bracts do not reflex
Wax on the bract	Moderately waxy	Few waxy	Moderately waxy	Very little or visible sign of wax	Very little or visible sign of wax	Very waxy
Presence of grooves on the bract	Moderately grooved	Few grooved	Strongly grooved	Moderately grooved	Few grooved	Strongly grooved
Male flower behaviour	Falling after the bract	Falling after the bract	-	Falling after the bract	Falling after the bract	Falling before the bract
Compound tepal basic color	Cream	Yellow	Yellow	Yellow	Cream	Yellow
Compound tepal pigmentation	Very few or visible sign of pigmentation	Very few or visible sign of pigmentation	Rust-colored spot	Very few or visible sign of pigmentation	Very few or visible sign of pigmentation	Presence of pink
Lobe color of compound tepal	Yellow	Yellow	Yellow	Yellow	Cream	Yellow

Lobe development of compound tepal	Developed	Little or visible of development	Developed	Little or visible of development	Developed	Little or visible of development
Free tepal color	Tinted with Yellow	Tinted with Yellow	Opaque white	Translucent white	Opaque white	Translucent white
Free tepal shape	Rounded	Fan-shaped	Rounded	Rounded	Rounded	Oval
Free tepal appearance	Corrugated below the tip	Corrugated below the tip	Corrugated below the tip	Corrugated below the tip	Corrugated below the tip	Not corrugated below the tip
Free tepal apex development	Very development	Developed	Very development	Very development	Little or no visible sign of development	Developed
Free tepal apex shape	Triangular	Triangular	Triangular	Triangular	Obtuse	Triangular
Filament color	White	White	Yellow	White	Cream	White
Anther color	Cream	White	White	Cream	Cream	Yellow
Polen sac color	Brown	brown	White	Cream	Cream	Yellow
Style Basic color	White	Cream	Cream	White	Cream	White
Pigmentation on style	No pigmentation	No pigmentation	No pigmentation	No pigmentation	No pigmentation	No pigmentation
Style shape	Straight	Curved at the base	Curved at the base	Straight	Straight	Straight
Stigma Color	Cream	Yellow	Yellow	Cream	Cream	Yellow
Ovary shape	Arched	Arched	Arched	Arched	Arched	Straight
Ovary Basic Color	Green	Yellow	Green	White	Green	Cream
Ovary pigmentation	Very few or no visible sign of pigmentation	Very few or no visible sign of pigmentation	Very few or no visible sign of pigmentation	Very few or no visible sign of pigmentation	Very few or no visible sign of pigmentation	Very few or no visible sign of pigmentation
Arrangement of ovules	Two rowed	Two rowed	Four rowed	Two rowed	Two rowed	Four rowed
Fruit position	Curved upward	Perpendicular to the stalk	Perpendicular to the stalk	Curved towards stalk	Curved upward	Perpendicular to the stalk
Number of fruit	13-16	13-16	≤ 12	≥ 17	≥ 17	≤ 12
Fruit length	16-20 cm	≤ 15 cm	≥ 31 cm	16-20 cm	≤ 15 cm	≤ 15 cm
Fruit shape	Curved	Curved	Curved	Straight in the distal part	Curved	Straight in the distal part
Transverse section of fruit	Rounded	Slightly ridged	Pronounced ridges	Slightly ridged	Rounded	Pronounced ridges
Fruit apex	Lengthily pointed	Lengthily pointed	Lengthily pointed	Blunt-tipped	Bottle-necked	Blunt-tipped
Remains of flower relict at fruit apex	Persistent style	Persistent style	Not persistent	Not persistent style	Not persistent style	Persistent style
Fruit pedicel length	≥ 21 mm	≤ 10 mm	≥ 21 mm	≤ 10	≤ 10 mm	≥ 21 mm
Fruit Pedicel width	5-10 mm	5-10 mm	≥ 10 mm	5-10 mm	5-10 mm	≥ 10 mm
Pedicel surface	Hairless	Hairy	Hairless	Hairy	Hairy	Hairless
Fusion of pedicle	Very partially or no visible sign of fusion	Very partially or no visible sign of fusion	Very partially or no visible sign of fusion	Very partially or no visible sign of fusion	Partially fused	Very partially or no visible sign of fusion
Immature fruit peel color	Light green	Green	Light green	Green	Green	Dark green
Mature fruit peel color	Orange	Bright yellow	Yellow	Light green	Yellow	Yellow
Fruit peel thickness	≤ 2 mm	≤ 2 mm	≥ 3 mm	≥ 3 mm	≤ 2 mm	≥ 3 mm
Adherence of the fruit peel	Fruit does not peel easily	Fruit peel easily	Fruit does not peel easily	Fruit peel easily	Fruit peel easily	Fruit does not peel easily
Cracks in fruit peel	Without cracks	Without cracks	Without cracks	Cracked	Cracked	Without cracks
Pulp in fruit	With pulp	With pulp	With pulp	With pulp	Without pulp	Without pulp
Pulp color before maturity	White	White	White	White	White	Cream
Pulp color at maturity	Orange	Cream	Ivory	Cream	Yellow	Cream
Fruit fall from hands	Persistent	Deciduous	Persistent	Deciduous	Deciduous	Persistent
Flesh texture	Soft	Soft	Firm	Soft	Soft	Firm
Predominant taste	Sweet	Sweet	Astringent	Sugary	Sweet	Astringent
Presence of seed with source of pollen	No seed	No seed	No seed	No seed	≥ 20	≥ 20





**Figure 1.** Morphological characters of six banana cultivars from East Kalimantan. A. Liar/monyet banana (*M. acuminata*), B. Klutuk banana (*M. balbisiana*), C. Talas banana, D. Rutai banana, E. Ambon banana, F. Tanduk banana

### Flower morphology and fertility status

The flower morphology can be used as an indicator of the banana sterility determining seed production in the fruit and might be the genomic ploidy of the plants. The flower morphology and physiology are one of the factors determining the gamete sterility besides the chromosome structure such as heterozygosity and triploidy causing meiotic error during gamete cell division (Bakry et al. 2009). The fertile banana plant is shown by the seed occurrence in the fruit indicating the successful fertilization of the banana flower. Most modern bananas are seedless

which have triploid genome ( $3n$ : AAA, AAB, ABB, or BBB) since they are male sterile. The triploid is the optimum ploidy that can produce good agronomic performance and shows gamete sterility. The triploid banana occurs due to the crossing fertilization between the diploid banana producing  $2n$  gametes and other diploid species producing  $n$  gamete (Bakry et al. 2009). The fertile bananas produce seeds in the fruit mostly derived from the diploid or tetraploid cultivars ( $2n$ : AA, BB or  $4n$ : AABB, BBBB, AAAB, and ABBB) although the number of seeds is varied among accessions. The fruit development of



banana is contributed by the parthenocarpy process which allows the development of fruit without fertilization process (Dodds and Simmonds 1948). It is contributed mostly from the *M. acuminata* character (Simmonds 1953). The selection of cultivated banana is based on the parthenocarpy, gamete sterility, and triploidy (Simmonds and Shepherds 1955).

The flower morphology observation of liar/monyet, Ambon, rutai, talas, tanduk, and klutuk banana showed that liar/monyet and klutuk bananas are fertile of which flowers contained functional pollens (Figure 2.A and F). This indication is supported by the seed existence composing the fruit aril in the liar/monyet and klutuk banana (Figure 2.A and F). Liar/monyet banana is reported as a diploid banana which has AA genome (Retnoningsing 2009; Retnoningsing et al. 2014) and klutuk banana (*M. balbisiana*) is a diploid banana containing BB genome (Sumardi and Wulandari 2010; Retnoningsing et al. 2014; Fitriyah et al. 2017). Therefore the production of seeds in their fruit aril is supported by the functional and fertile flowers of the diploid banana cultivars. On the contrary, the pollens of Ambon, rutai, talas, and tanduk banana flowers are absent (*male sterile*). These indicate that the most

important organ for fertilization is not functional therefore the seeds are absent in their fruit (Figure 1.B, C, D, and E). However, the structure of anther, stigma, stylus, and tepal of Ambon, rutai and talas banana is complete and normal. The only abnormal organ was the pollen absence (Figure 2.B, C, D). Whereas, the organ structures of tanduk banana supporting the flower are abnormal, truncated, and even absent (Figure 2.E). Ambon banana has been identified as a triploid with AAA genome (Retnoningsing et al. 2014), so the male sterility is contributed by its genome ploidy ( $3n$ ) resulted in the seedless fruit. The genome group classification of rutai and talas banana has not been identified yet, but from the flower morphology and seed composition in the fruit, they could be suggested as triploid bananas. The abnormal flower structure of tanduk banana might contribute to the seedless and abnormal structure of the tanduk banana fruit (Figure 1.F). The genome group classification of tanduk banana has been reported as a triploid with AAB genome (Retnoningsing et al. 2014), but it should be verified since the flower morphology is quite different (Figure 2.E), therefore the sterility could be due to the genome instability or change and might not be because of the ploidy (triploid/ $3n$ ).



**Figure 2.** Flower morphology of six bananas from East Kalimantan. A. Liar/monyet banana (*M. acuminata*), B. Ambon banana, C. Rutai banana, D. Talas banana, E. Tanduk banana, F. Klutuk banana (*M. balbisiana*)

### Genome classification

The genome classification scoring of the six banana cultivars was conducted based on the Simmonds and Shepherd (1955) system using 15 key characters (Table 4). Score 1 represented the character similar to *M. acuminata*, whereas score 5 represented characters from *M. balbisiana*. The score 2-4 represented intermediate character between *M. acuminata* and *M. balbisiana*. The total score then was used to determine the genome group of the banana (Table 2). The genome of liar/monyet, klutuk and Ambon banana in this study confirmed the previous reports having AA, BB, and AAA genome, respectively. A new records for talas and rutai banana which are grouped into AAB genome. Surprisingly, based on our observation tanduk banana is grouped into AB genome, which is different from the previous publications (Table 5).

Liar/monyet banana was grouped in AA genome with a score total of 15 (Table 5). This is a perfect match with *M. acuminata* characters with score 1 at all fifteen characters described (Table 4) and in line with the scoring category described in Table 2. Liar/monyet banana is most likely has AA genome, not AAA Genome, since the morphological observation showed that its fruits were fully seeded (Table 3, Figure 1.A) indicating a diploid clone. Furthermore, klutuk banana was categorized as BB genome since the total score of genome classification was 75 (Table 5) with score 5 at all fifteen key characters (Table 4) according to the genome group classification as described at Table 2. The genome is more exactly matched with BB genotype, since it has many seeds in its fruits (Table 3, Figure 1.B). These findings are in line with the previous study that included liar/monyet banana in AA genome and klutuk banana in BB genome (Retnoningsih 2009; Retnoningsing et al. 2014; Fitriyah et al. 2017).

Ambon banana was categorized in AA/AAA genome group since the total score in the genome classification was 19 (Table 5). Although most of the key character scores were similar to those belonging *M. acuminata*, there were some characters showed score more than 1 (Table 4). From the morphological observation, Ambon banana showed strong indication as triploid genotype with the indication of seedless fruit character and flower morphology (Table 3, Figure 1.E, Figure 2.B), therefore it leads the conclusion that Ambon banana is a triploid banana with AAA genome as previously reported by Retnoningsih et al (2014).

Two local cultivars of banana from East Kalimantan, talas and rutai, are included into AAB genome group (Table 5) with the total score of 31 and 28, although the score of each key character was varied (Table 4). From the scoring, there was a strong indication that both banana cultivars belong to the AAB genome and closer to the *M. acuminata* ancestor rather than *M. balbisiana*. Talas and rutai can be used as dessert/sweet banana, but only talas banana can be used as cooking banana. The seedless fruit (Table 3) and flower morphology (Figure 2.C and D) support the triploid characters of these banana genotypes. The triploid chromosome structure, sterile gamete, and the parthenocarp fruit development that has been revealed in these bananas are the most wanted characters for modern cultivated banana (Simmonds and shepherds 1955; Heslop-

Harrison and Schwarzacher 2007) and the goals of banana breeding (Brown et al. 2017). Since there is no previous study conducted to analyze the genome group of the talas and rutai banana, this result can be used as the first reference for sequential study using both clones. The interesting new finding was reported here that tanduk banana was classified as an amphidiploid banana with AB genome (Table 5). The score of each key characters in the genome classification scoring was varied and the total score was 48 (Table 4). Tanduk banana is an amphidiploid banana referring to banana containing two sets of chromosome derived from two different species. The fact that tanduk banana is an amphidiploid is supported by the unique morphology of the fruit (outward curved fruit), seedless fruit and abnormal flower morphology (Figure 1.F and 2.E). The unique morphological structure observed in amphidiploid banana was also reported in *Mchare* banana from Tanzania (Simmonds 1962). The existence of AB genotype is rare and some of the edible AB clones are found in south India, i.e., *Net Poovan* and *Kuman* and in Papua New Guinea (De Langhe et al. 2010). The amphidiploid genome, like AB, is usually sterile due to the mismatch chromosome pairing during meiosis resulting in an unequal chromosomal allocation at meiosis (D'Hont et al. 2000; De Langhe et al. 2010). The amphidiploid AB occurs due to the interspecific crossing between AA dan BB genome.

The genome classification of Indonesian bananas based on the morphological characters has been conducted for 267 accessions (Retnoningsih 2009), 20 accessions mainly from Sumatra (Rinaldi et al. 2014) and 30 accessions mainly from East Java (Fitriyah et al. 2017). The same result of genome classification between the previous studies and this current study were observed at the liar/monyet, Ambon, and klutuk banana, whereas, for the tanduk banana, the different result was found. Tanduk banana has been classified as AAB genotype in the previous study, but it is classified as AB genotype in this study. The finding that tanduk banana is classified as AB genotype will give new record for the existence of AB accessions. The subsequent study is suggested using other strategies such as molecular and/or cytogenetic analysis for clarification.

### Diversity analysis

The diversity analysis using morphological character was conducted to figure out the genetic relationships of talas and rutai banana with the common identified bananas, i.e., liar/monyet (*M. acuminata*), klutuk (*M. balbisiana*), Ambon and tanduk banana.

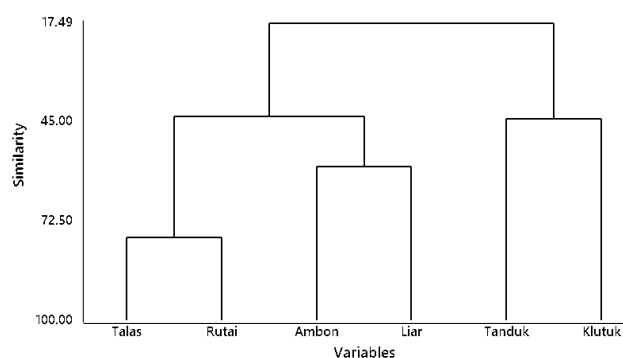
The banana accessions are grouped into two main clusters (Figure 3). The first cluster composed of the *balbisiana*-group which comprises of BB genotype (klutuk banana/*M. balbisiana*) and AB genotype (tanduk banana). The second cluster is *acuminata*-group comprising two subgroups, namely, AA/AAA and AAB genotype (Figure 3). The AA/AAA subgroup is composed of liar/monyet banana (AA genome) and Ambon banana (AAA genome). Another subgroup is AAB genotype bananas comprising talas and rutai banana.

**Table 4.** Genome group scoring of six banana cultivars from East Kalimantan, Indonesia

Character	Talas	Rutai	Tanduk	Ambon	Liar/monyet	Klutuk
Pseudostem color	2	3	4	2	1	5
Petiole canal	1	1	5	1	1	5
Peduncle	2	2	5	2	1	5
Pedicels	5	4	5	1	1	5
Ovules	1	1	5	1	1	5
Bract shoulder	2	2	1	1	1	5
Bract curling	1	1	4	1	1	5
Bract shape	3	1	1	1	1	5
Bract apex	2	1	1	1	1	5
Bract color	3	3	2	2	1	5
Color fading	3	3	2	1	1	5
Bract scars	1	1	4	1	1	5
Free tepal of male flower	1	1	1	1	1	5
Male flower color	2	1	4	2	1	5
Stigma color	2	3	4	1	1	5
Total score	31	28	48	19	15	75

**Table 5.** Genome group classification of six bananas from East Kalimantan, Indonesia

Banana cultivar	Total score	Genome classification
Liar/monyet ( <i>Musa acuminata</i> )	15	AA
Talas/sunking	31	AAB
Rutai	28	AAB
Ambon	19	AAA
Tanduk	48	AB
Klutuk ( <i>Musa balbisiana</i> )	75	BB

**Figure 3.** Dendrogram of six banana accessions from East Kalimantan, Indonesia (presented in % of similarity)

The similarity between two main groups, i.e., *acuminata*-group and *balbisiana*-group was approximately 17.49%. Furthermore, the highest level of similarity was observed between talas and rutai banana about 75%,

followed by Ambon and liar/monyet (about 48%) and tanduk and klutuk (about 45%). The results of diversity analysis were in line with the results of the genome group classification and morphological observation as previously presented (Table 1 and 5, Figure 1 and 2). The AAB genotypes (talas and rutai banana) revealed in this genome classification (Table 5) were much closely related to *acuminata*-type in diversity analysis. The same result was observed in AA/AAA genome accessions (Ambon and liar/monyet banana) which were also genetically close related. Furthermore, between AB and BB genotype (tanduk and klutuk banana) were clustered in the same group (Figure 3), although the similarity level was lower (45%).

The genetic relationships and diversity among banana accessions have been extensively studied using morphological characters (Heslop-Harrison and Schwarzscher 2007; Sukartini 2007; Rinaldi et al. 2014; Fitriyah et al. 2017), RAPD markers (Kaemmer 1992; Howell et al. 1994; Jain et al. 2007), RFLP markers (Gawel and Jarret 1991; Jarret et al. 1992; Nwakanma et al. 2003; Raboin et al. 2005), SSR/ISSR markers (Creste et al. 2006; Retnoningsih 2009; Qin et al. 2011), AFLP markers (Wong et al. 2002; Noyer et al. 2005; Wongniam et al. 2010), microsatellite markers (Wahyuningtyas et al. 2009), ITS and IRAP Markers (Pachau et al. 2014), and sequencing (Aert et al. 2004; Cheung and Town 2007; D'Hont et al. 2012; Davey et al. 2013). All genetic diversity analysis place *M. acuminata* and *M. balbisiana* in the different pool/group separated in very far genetic distance (low similarities) since they are from different species, whereas their interspecific-hybrids, referred as *M. paradisiaca*, are occupied in between of them (Espino et al. 1992). The existence of talas and rutai banana in the banana references will enrich genetic diversity pool of bananas contributing genetic sources for the banana breeding program and genetic improvement.

The morphological observation of around 108 characters showed that there is strong indication that characters of talas and rutai bananas are much closed related to those of belonging to *M. acuminata* although some characters showed *balbisiana*-type. In addition, as reported in many studies, liar/monyet and klutuk banana morphological characters represented the *M. acuminata* and *M. balbisiana* accessions. Furthermore, Ambon banana was very closely related to *M. acuminata* with the higher plants and bigger fruit but tanduk banana had a combination characters between *M. acuminata* and *M. balbisiana*. The genome group classification showed that talas and rutai banana are the AAB genotype, while Liar, Ambon, tanduk, and klutuk were grouped into AA, AAA, AB, and BB genotypes, respectively. The classification of AB genome for tanduk banana was a new finding after it was reported as AAB genotype in previous studies. The diversity analysis showed that talas and rutai banana have very high similarities about 75% and closed related to AA/AAA genotype, i.e., liar/monyet and Ambon with the similarity level of 45%. The similarity of *acuminata*-type group and *balbisiana*-type group in this study was around 18%.

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