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Anatomy and morphology character of five Indonesian banana cultivars (*Musa* spp.) of different ploidy level

ISSIREP SUMARDI♥, MERA WULANDARI
Faculty of Biology, Gadjah Mada University (UGM), Jl. Teknika Selatan, Sekip Utara, Sleman, Yogyakarta 55281, Indonesia. Tel. & Fax: +62-0274-580839, ♥email: issirepsumardi@yahoo.co.id

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ABSTRACT

*Sumardi I, Wulandari M (2011) Anatomy and morphology character of five Indonesian banana cultivars (*Musa *spp.) of different ploidy level. Biodiversitas 12: 167-175.* In Indonesia there are many cultivars of banana, and some of them produce edible fruits. Beside their morphology, the character which necessary as a tool for classification is anatomical character. The aim of this research were to describe the anatomical character and morphology of fives Indonesian banana cultivars based on their level of ploidy. The cultivars were collected from Banana Germplasm Plantation, Yogyakarta District, Indonesia. The samples of roots, rhizome, and leaf were collected from five banana cultivars i.e.: *Musa acuminata* cv Penjalin, *M. balbisiana* cv Kluthuk warangan, *M. acuminata* cv Ambon warangan, *M. paradisiaca* cv Raja nangka, and *M. paradisiaca* cv Kluthuk susu. For anatomy observation samples were prepared using paraffin method, stained with 1% safranin in 70% ethanol. To observe the structure of stomata and epidermis surface, slide were prepared using modification of whole mount method. Slides were observed using Olympus BHB microscope completed with Olympus camera BM-10A. Stem and leaf morphology character of diploid level (AA and BB genome) is different with triploid level (AAA, AAB, and ABB genome). Anatomy and morphology character of root and rhizome of banana in diploid level (AA and BB genome) and triploid level (AAA, AAB, and ABB genome) is quite similar. Distribution of stomata is found in leaf and pseudostem. Stomata is found in adaxial and abaxial epidermis layer. The size of guard cells in triploid cultivars was longer than that diploid cultivars. The root composed of epidermis layer, cortex and cylinder vascular of five cultivar's root show anomalous structure. Rhizome consist of peripheric and centre zone. Anatomically, this was no differences in the rhizome structure among five banana cultivars. The row of vascular bundles acts as demarcation area between peripheric and central zone. In the cultivar with BB genome (diploid) and ABB genome (triploid) the row of vascular bundle was not found. The differences of leaf anatomy were base on: size and number of stomata distribution, number of subsidiary cells, number of hypodermal layers, structure and number of parenchyma palisade, size of airspace in petiole and mesophyll and the vascular bundle structure.

Key words: anatomical character, morphology, banana cultivar, ploidy level.

INTRODUCTION

Bananas are among the largest herbs in the world. They are perennials with tall aerial shoots that arise from swollen, fleshy corms. The distribution of species is influenced by morphology, chromosome number and geographical location (Wang et al. 2010). Nowadays the existing banana in many countries was supposed as a line of *Musa acuminata* Colla and *M. balbisiana* Colla (Simmonds 1959). The line species are diploid (AA genome), triploid (AAA genome) and tetraploid (AAAA genome). Banana plants have various ploidy level, as a result of natural crossing between wild species continuously and the effect of environment. These process cause the rise of new species with different ploidy level, i.e.: diploid, triploid and tetraploid. Crossing between *M. acuminata* (AAAA genome) and *M. balbisiana* (BB genome), for example, was resulting triploid level with genome symbol AAB or ABB (Purseglove 1979). Caryotype and number of chromosome are generally very importance in studying classification, but the chromosome number is not absolutely as a case, because some species of plant that have same chromosome number perform different character. *M. acuminata* (AA genome, 2n=22) produce edible fruit, but *M. balbisiana* (BB genome, 2n=22) has many seed and not edible fruit (Fitri 2007).

Cheung and Town (2007) reported in order to view the sequence composition of the *M. acuminata* in a cost effective and efficient manner, 6.252 of BAC (Bacterial Artificial Chromosomes) gene sequences were search again several data bases, and significant homology was found in mitochondria, chloroplast, and protein. Wang et al. (2010) compare 20 sugarcanes BAC with sorghum sequencing to know the character of complex autopolyploid sugarcane at the DNA sequencing level. The complexity of the autopolyploid genome at the interspecific hybridization of modern cultivar hinders progress in genetic research and the application of genomic tool in breeding program (D'Hont 2005). Recent genome and molecular cytogenetic data provided cytogenetic evidence that some species were derived from interspecific hybridization between two different species (D'Hont et al. 2002).

The line species from *M. acuminata* and *M. balbisiana* crossing is *M. paradisiacal* Linn. Biodiversity of banana

cultivar are caused by natural crossing or somatic mutation proceeds for along time (Stover and Simmonds 1987) or caused by the selection and vegetative propagation (Purseglove 1979). The diversity of banana could be differentiated by the taste, shape and color of fruit. The species and cultivar of banana which to be found in Indonesia have not all been classified yet. Molecular approach and chromosome caryotype have been used to determine the phylogenetic relationship among some species of bananas (Retnoningsih 2009). Kustanti (2005) studied the relationship of Belo bamboo with three bamboo genera based on their stem anatomy. Wardhana et al. (2009, pers. comm.) worked with some species of yams tuber to determine their relationship based on tuber anatomy. Anatomy traits were selected from previous report of phase-specific in maize (Boungard-Pierce et al. 1996) and in three grasses plants: maize (*Zea mays* L.), rice (*Oryza sativa* L.) and bluegrass (*Poa pratensis* L.) (Sylvester et al. 2001). The traits included epidermis cell shape, the presence and absence of epicuticular wax, hairs, stomata and bulliform cell.

Anatomy knowledge is essential when vegetative propagation is used to identify important structural feature necessary for propagation success (Silva-Lima et al. 2005). Information on anatomical structure is needed by breeder working on improvement for drought tolerance (Nassar et al. 2008). Using 0.2% colchicine they found various ploidy levels in cassava plant. Tetraploid type in cassava show more prismatic and druse crystal in the cortical parenchyma, pericycle fibers had thickening wall, secondary xylem was wider than diploid one, which having thinner walls and less starch.

The aims of this research were to examine the morphological and anatomy characters of five Indonesian banana cultivars found in Yogyakarta based on their ploidy level. This character is very important as supporting data for classification.

MATERIAL AND METHODS

Plant materials. Samples were collected from Banana Plantation in Yogyakarta District, Indonesia. The five cultivars were: *M. acuminata* cv Penjalin (AA genom), *M. balbisiana* cv Kluthuk warangan (BB genom), *M. acuminata* cv Ambon warangan (AAA genom), *M. paradisiaca* cv Raja nangka (AAB genom) and *M. paradisiaca* cv Kluthuk susu (ABB genom). The morphological character included root, rhizome, pseudostem, leaf and plant habit. For anatomical characters: root, rhizome, pseudostem, leaf blade and midrib, shape, size and distribution of stomata in epidermis layer and pseudostem were observed.

Morphology. Morphology characters observed were: root, growth of adventitious root, rhizome color, pseudostem color, leaf blade, shape and size of midrib and plant habitus.

Anatomy. In order to analyze of vegetative structures, samples (root, rhizome, leaf blade, petiole, and pseudostem) were prepared using paraffin method, while epidermis layer of leaf and pseudostem were processed using modification of whole mount method (Ruzin 1999).

Paraffin method procedure. Material was fixed in FAA (90 mL ethanol 70%, 5 mL acetic acid, and 5 mL formaldehyde 36%) solution; (ii) the materials were washed repeatedly in 70% ethanol and dehydrated with ethanol series (80%, 90% and 95%); (iii) sequently dealcoholization step using absolute ethanol and xylene mixture i.e.: ethanol/xylene 3: 1; 1: 1; and 1: 3 (iv) infiltration step: the mixture of ethanol/xylene was replace with the mixture of liquid paraffin and xylene (9: 1), for 24 hours; (v) embedding step: before embedding step, the material was immersed in pure liquid paraffin for one hour and then embedded using the pure paraffin ($56^0/57^0$C); (vi) The embedded sample were sectioned in thinly slide using rotary microtome. The sliced sample were stained with 1% safranin in 70% ethanol; (vii) after staining, the slides were observed under the Olympus microscope and the photograph were taken using the Olympus BHB Model completed with Olympus Camera BM-10A.

Whole mount method. the peeling of leaf epidermis layer and pseudostem each were immersed in chloral-hydrate (250 g/100 mL) for short time until the material became transparent; (ii) the samples were then washed two to three times in distilled water to remove the trace of chloralhydrate, and stained the material with 1% safranin in distilled water for 20 minutes; (iii) the material were washed repeatedly with distilled water; (iv) the materials then were put in slideglass and small drops of the glycerine were added to the slides and covered with coverslip; (v) the slides were observed under Olympus microscope and photograph were made using microscope like above

RESULT AND DISCUSSION

Morphology

Root of the five cultivars perform quite similar in morphology character behavior (Table 1). The young roots showed white color, and became brown in mature roots. Many root hairs were found on the surface of root.

Stem of the five cultivars were different in stem diameter and pseudostem color. The true stem was formed when the plant started to form reproductive organ. The pseudostem was formed as the modification of the lower part of the midrib. Pseudostem had red, yellowish green and reddish green color. On the basal part of the stem big structure called rhizome was found.

Leaf of banana was belonging complete type group, because they had midrib, petiole and leaf blade. The modification of basal part of midrib was called pseudostem. The petiole had halfcircle-like shape and the adaxial part grooved. The shape of leaf blade was oblong with flat tip. The abaxial and the adaxial surface of leaf were protected by cuticle layer. The cutin layer was also found in the petiole. The five cultivars studied formed green leaves with nearly the similar size.

General anatomy

Root of all five cultivars has similar structure, which consist of three tissue systems, epidermis, ground parenchyma and vascular cylinder.

Cortex. of mature roots had many layers with thick-walled cells in outside surface. According to Tomlison (1969) those tissues were periderm. This tissue was a protected layer. Young root of cultivar Penjalin and Raja nangka have one to two epidermis layers. Cortex composed of parenchyma cells, with many and big airspace. The shape of parenchyma cells was irregular. The big airspace became larger in size. Swennen and Oritz (1997) called it lacunae. The large airspace in several species of monocotyledon may have schizogenous or lysigenous in origin. But sometime it may arise by combination of the two processes (Esau 1978). Airspace of some roots is regarded as a serving tissue in gas transport, because airspace as reservoir of oxygen, which is required in the respiration by the tissue which have no access to the oxygen of the air (Fahn 1990). From five cultivars, only Raja nangka cultivar has no airspace.

Endodermis. the boundary layer between cortex and vascular cylinder, consist of one layer which composed of thick cell. The cell wall of endodermis has U-like shape thickening (Esau 1978; Raven et al. 1999). This wall composed of suberin and cellulose. At first thickness of the endodermis was like strip, and then developed into a band, called Casparian band (Esau 1978). The endodermis cells continuously thickened, and finally the shape of band changed to the U shape. Suberin and cellulose materials deposited radially and tangensially in inner side wall. This condition was found in Penjalin, Kluthuk warangan, Ambon warangan and Kluthuk susu cultivar, while the structure of endodermis in Raja nangka cultivar was not similar with the others.

Pericycle. the outer layer of vascular cylinder was called pericycle. It was a single layer, and composed of meristematic cells. In mature root the function of this layer was to form adventitious roots (Fahn 1990). Some root of five cultivars showed primordial of adventitious root which originated from the pericycle. For example, roots of Penjalin cultivar, Ambon warangan, and Raja nangka.

Vascular cylinder (stele). Generally vascular system in monocotyledons is radial. In banana the vessels scattered at the center of root. The phloem cells were formed alternately with vessel cells in the periphery side of cylinder, and no pith was found. Generally in monocotyledonous root, xylem frequently forms a solid core with ridgelike projections, and strands of phloem alternate with the xylem ridge (Esau 1978). In banana root, phloem widespread between the vessels in the center. In root of Kluthuk warangan, some of vessels were surrounded by tracheid cells. So the arrangement of phloem irregular in the central zone, and xylem did not form ridge-like structure as found generally in monocotyledonous root. This condition showed the anomalous structure (Figure 1). This result contributed by Tomlison (1969) supposed, that the development of banana root showed anomalous structure. According Swennen and Oritz (1997) the formation of xylem in banana root will stop when the root stop to elongate. Root parenchyma composed of thick wall cells.

Laticifer. Laticifer were scattered in the cortex and vascular cylinder zone of five banana cultivars root. Laticifers are cells or series of connected cells that contain latex, a fluid or complex composition substances. Banana laticifer was clearly colorless, like milk or brown color. In Musaceae, laticifer the compound type which were derived from series of cells. The series of cells in compound laticifer become united by dissolution of intervening walls. By this junction the laticifer cells compound developed wall perforation and this structure was called articulate (Nugroho et al. 2003). The type of laticifer in all banana cultivars was compound type and non anastomosis. The differences of five cultivars root can be seen on Table 1.

Anatomy of rhizome and pseudostem

Cross section

Rhizome. The basal part of banana stem is rhizome. Rhizome grew after reproductive organ were formed as modification of the peduncle, white in color, with smooth surface. Rhizome consists of epidermis, periphery zone and center zone.

Epidermis. It was a single layer, dense cells, without intercellular space. In mature rhizome, many layers of periderms were found beneath the epidermis. In rhizome there was no cortex like those in monocotyledons stem. In Monocotyledonous stem there are no cortex as well as stele because no demarcation between both area (Nugroho et al. 2006; Esau 1978). Rhizome of the five banana cultivars consist of two zones, i.e.: periphery zone and central zone.

Pheriphery zone. It consisted of parenchymatic cells and small vascular bundles, with no partition between central zone. The shape of parenchymatic cells in periphery was irregular and small. The periphery zone was narrower than central zone. Small vascular bundle were scattered in the periphery zone. The xylem of vascular bundle consists of vessel cell and not all of bundle was protected by thick wall tissues.

Central zone. It consisted of irregular parenchymatic cells, which was wider compared with the periphery zone. Vascular bundle were scattered and their number were quite frequent. The partition between central and peripheric zone generally marked by the row of vascular bundles which made them close regularly. The type of vascular bundle was close-collateral, because no cambium layer between xylem and phloem were formed. The size of vascular bundle in central zone was bigger than in the periphery zone. In the part of the central zone the vascular bundles were even bigger. The xylem consists of vessel only, with thick wall. The phloem with thin wall, gathered in the side of vessel cells. The bundle was not protected by sheath. The size, structure, and diameter of vascular bundle were varied among five banana cultivars, and these variations were depended on the ploidy level. Cytological work by de Azkue and Martinez (1990) found a group of a dozen morphologically similar Andean species that share a base of chromosome number of x=8 which is rare in *Oxalis*. Base of chromosome numbers *Oxalis* vary from x=5 to x=12, with x=7 most frequent, and polyploidy is common in the genus (Emshwiller and Doyle 1998).

Laticifer. Laticifer was found in rhizome of five banana cultivars. Laticifers were rounded by the parenchymatic cells. The structure of rhizome laticifer was similar with that of root laticifer. The location of laticifer cells were

Table 1. Root and rhizome morphology and anatomy characters of five Indonesian banana cultivars of different ploidy level

Character	Cultivar				
	Penjalin (AA)	**Kluthuk warangan (BB)**	**Ambon warangan (AAA)**	**Kluthuk susu (ABB)**	**Raja nangka (AAB)**
Root					
Morphology					
▪ Root type	Fibrous	Fibrous	Fibrous	Fibrous	fibrous
▪ Root color	Young: white Mature: brown	Young: white Mature: brown	Young: white Mature: brown	Young: white Mature: brown	Young: white Mature: brown
Anatomy					
Epidermis					
▪ Number	1-2 layers	1 layer	1 layer	1 layer	1-2 layers
Cortex					
▪ Shape of parenchyma	4-8 sides	Irregular	Irregular	Irregular-four sides	4-8 sides
▪ Airspace	Radial	radial	Radial	Radial	No airspace
▪ Pericycle	1 layer	1 layer	1 layer	1 layer	1 layer
Cylinder vascular					
▪ Xylem	Protoxylem in periphery, metaxylem scattered	protoxylem and meta-xylem were rounded by thick wall cell	protoxylem was rounded by thick wall cell; metaxylem scattered	Protoxylem in periphery; metaxylem scattered	Protoxylem in periphery; metaxylem scattered
▪ Phloem	Thick wall, scattered	Thick wall, scattered	Thick wall, scattered	Thick wall, scattered	Thick wall, scattered
▪ Stele	Anomalous	Anomalous	Anomalous	Anomalous	Anomalous
Laticifer					
▪ Distribution	Cortex and stele	Cortex and stele	Cortex and stele	Cortex and stele	Cortex and stele
▪ Type	Non anastomosis	Non anastomosis	Non anastomosis	Non anastomosis	Non anastomosis
Rhizome					
Morphology					
▪ Color	White	White	White	White	White
▪ Surface	Soft	Soft	Soft	Soft	Soft
Anatomy					
Epidermis					
▪ Number	1 layer	1 layer	1 layer	1 layer	1 layer
▪ Shape	Four sides	Four sides	Four sides	Four sides	Four sides
Periphery zone					
▪ Parenchyma shape	Irregular	Irregular	Irregular	Irregular	Irregular
Central zone					
▪ Xylem	1, trachea	1, trachea	1, trachea	1, trachea	1, trachea
▪ Phloem	One side of xylem	One side of xylem	One side of xylem	One side of xylem	One side of xylem
▪ Vascular bundle type	Close collateral	Close collateral	Close collateral	Close collateral	Close collateral
▪ Stele	Atactostele	Atactostele	Atactostele	Atactostele	Atactostele
Pith	No pith	No pith	No pith	No pith	No pith
Articulate latisifer	Near phloem	scattered	scattered	scattered	Near phloem

near the phloem tissue, and this was supposed to be articulate type. According to Fahn (1990) the articulate laticifer tubes developed in the phloem tissue of stem and contain of tannin.

The differences of anatomical characteristic of rhizome of five banana cultivar were presented in Table 1.

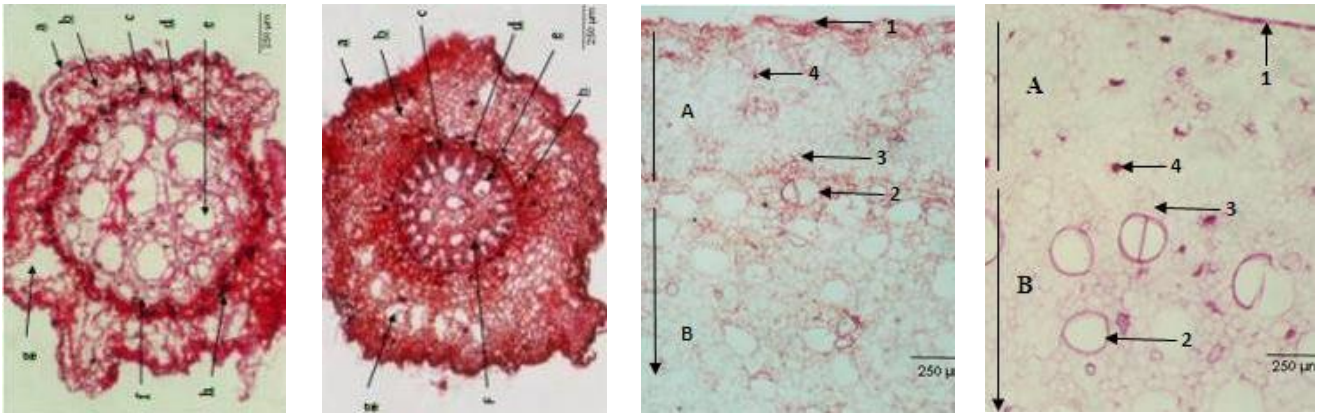
Paradermal section

Pseudostem. Actually the pseudostem of banana was the result of growth and development of the leaf midrib surrounding the rhizome. By peeling the pseudostem it was noted that the structure of epidermis (outer and inner) layer consisted of epidermis cells and stomata.

*Epidermis***.** The shapes of outer and inner epidermis cells were rectangular. The arrangements of epidermis cell were compact without inter-cellular space. According to Fahn (1990) the epidermis cells of monocotyledonous stem were stretched lengthwise. The sizes of inner epidermis cells were longer and wider than those of the outer one.

The longest and widest size of outer epidermis cells were found in Raja nangka cultivar (AAB genome) with average of 63.756±9.957 µm and 16.936±2.159 µm. The shortest size of outer epidermis cells was found in Kluthuk warangan cultivar (BB genome) which was 40.964±8.684 µm and Penjalin cultivar (AA genome) was 12.936±2.159 µm. The epidermis cells size of triploid cultivar were bigger than that of diploid cultivar. The traits of epidermis layer of three grasses plant had been observed by Sylvester et al. (2001).The trait of leaf epidermis layer in young and adult maize was distinctly, but similar differences were not found in rice and bluegrass leaf.

The longest and the widest size of inner epidermis was found in Kluthuk warangan (BB genome) with average of 71.7764±6.765 µm, and the widest one was Ambon warangan cultivar (AAA genome) with average of 36.344±5.924 µm. The shortest size of Kluthuk susu (ABB



1A **1B** **2A** **2B**

Figure 1. Cross section of root of *M. paradisiaca* cv. A. Kluthuk warangan (BB), B. Klutuk Susu (ABB). a, epidermis; b, cortex; c, endodermis; d, pericycle; e, vessel; f, phloem; g, air space; h, laticifer. Bar = 250 um.

Figure 2. Cross section of rhizome: I. *M. acuminata* cv. Penjalin (AA) II. *M. acuminata* cv. Ambon warangan (AAA). Note: A, periphery zone; B, centre zone; 1, epidermis; 2, vessel; 3, phloem; 4, laticifer. Bar = 250 um.

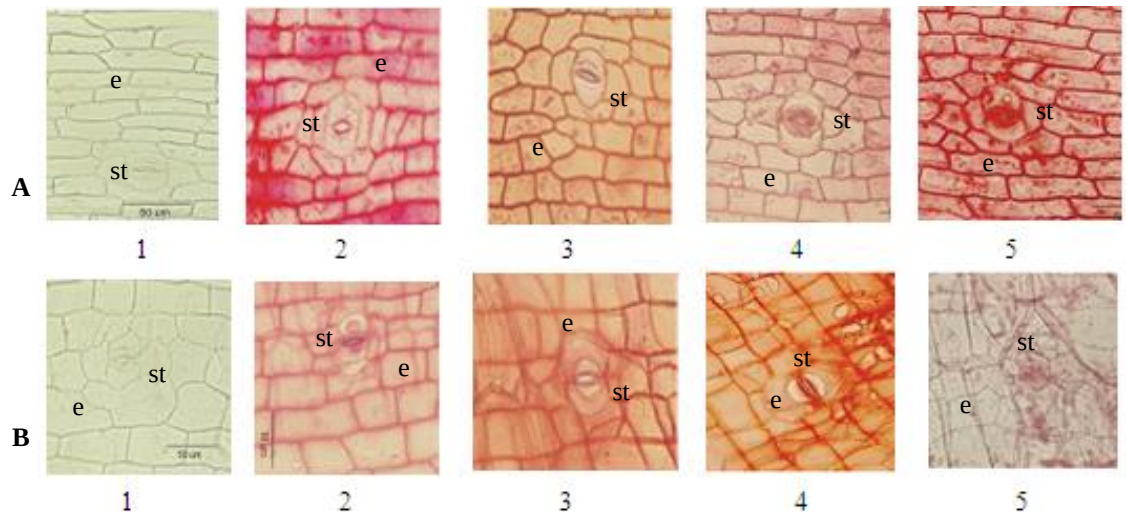
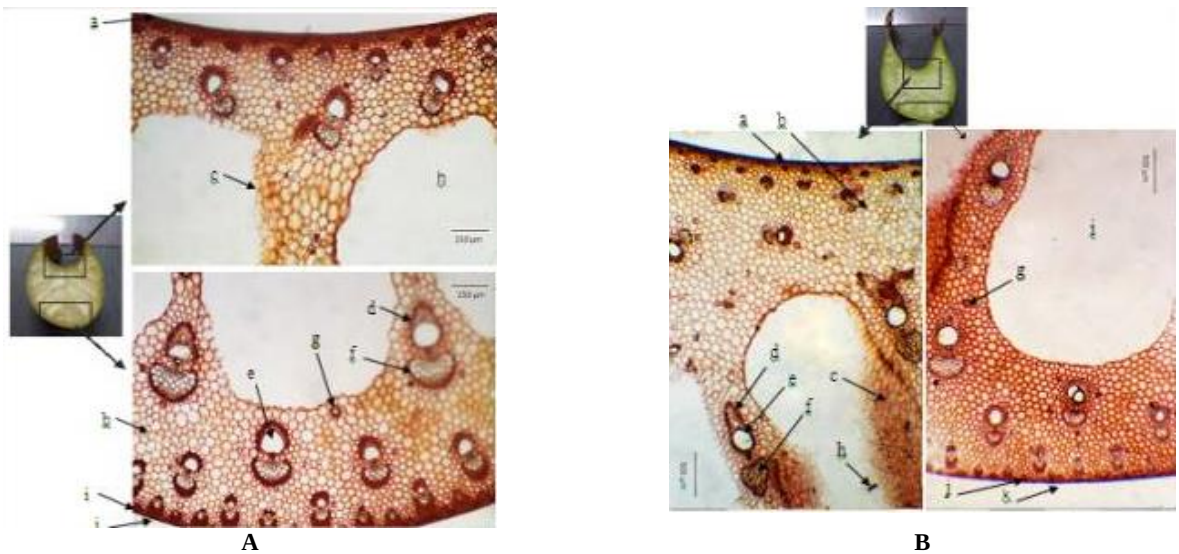


Figure 3. Epidermis layer of pseudostem of five banana cultivars. A. Outer layer; B. Inner layer. 1. *M. acuminata* cv. Penjalin (AA); 2. *M. acuminata* cv. Ambon warangan (AAA); 3. *M. balbisiana* cv. Kluthuk warangan (BB); 4. *M. paradisiaca* cv. Kluthuk susu (ABB); 5. *M. paradisiaca* cv. Raja nangka (AAB) e. epidermis cell; st. stoma. Bar = 50 um.



A **B**

Figure 4. Cross section of leaf petiole: A. *M. acuminata* cv. Penjalin (AA); B. *M. acuminata* cv Ambon warangan (AAA) a. epidermis; b. parenchyma cells; c. aerenchyma; d. sclerenchyma sheath; e. xylem; f. phloem; g. laticifer; h. air space; i. sclerenchyma; j. cuticle.

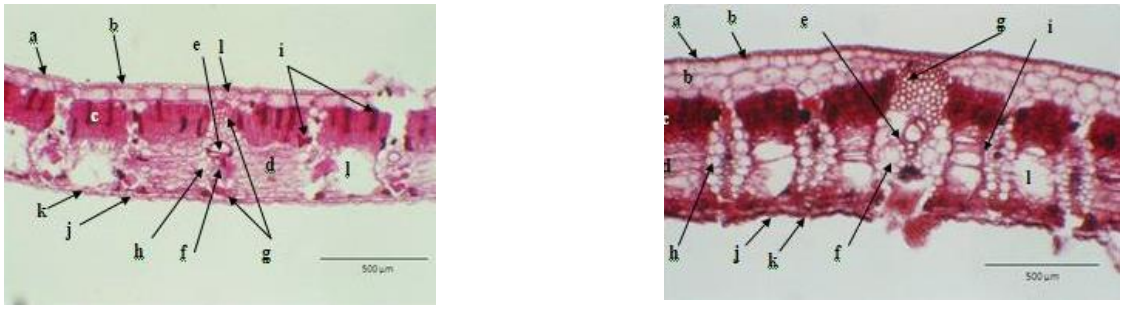


Figure 5. Leaf cross section: A. *M. balbisiana* cv. Kluthuk warangan (BB); B. *M. paradisiacal* cv. Kluthuk susu (ABB). a. adaxial epidermis; b. cuticle; c. palisade tissues; d. spongy tissue; e. xylem; f. phloem; g. sclerenchyma; h. bundle sheath; i. laticifer; j. abaxial epidermis; k. stomata; l. air space. Bar = 500 um.

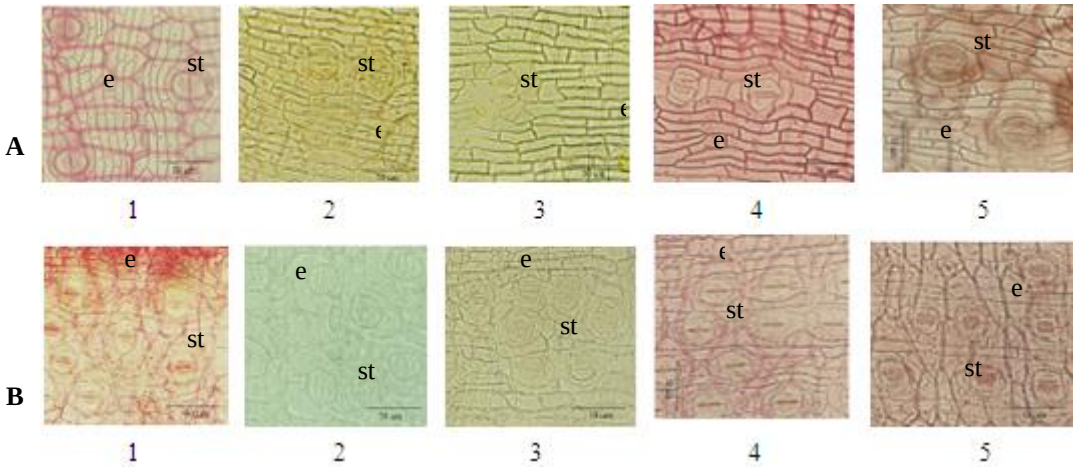


Figure 6. Leaf epidermis of five banana cultivars. A. Adaxial surface; B. Abaxial surface. 1. *M. acuminata* cv. Penjalin (AA); 2. *M. acuminata* cv. Ambon warangan (AAA); 3. *M. balbisiana* cv. Kluthuk warangan (BB); 4. *M. paradisiaca* cv. Kluthuk susu (ABB); 5. *M. paradisiaca* cv. Raja nangka (AAB). e. epidermis cell; st. stoma. Bar =50 um.

genome) with average of 53.9±5.553 µm, while the narrowest one was Kluthuk warangan's (BB genome) with average of 28.336±2.798 µm. It was showed from the result that the size of epidermis layer did not depend on the ploidy level. Statistical analysis showed that ploidy level had significantly affect on outer epidermis cell size of pseudostem. Suryo (2007) supposed that the higher level of ploidy, the bigger epidermis cells.

*Stomata***.** Stomata were located on inner and outer parts of pseudostem epidermis layer. The guard cells of stomata were kidney like, rounded by 4-6 subsidiary cells. This condition was in line with Fahn (1990) statement, that the Musaceae family had 4-6 subsidiary cells. The structure of the the subsidiary cell was quite similar to the epidermis cell around them, so the type of this stomata was called anomositic.

The stomata of Penjalin and Kluthuk susu cultivar were surrounded by 4-7 cells and 4-8 cells respectively. The distribution and the shape of stomata either in outer or inner epidermis layer were different. Statistical analysis showed that the ploidy level significantly affected to the length of the stomata in outer epidermis layer. Penjalin (AA genome) and Kluthuk warangan (BB genome) cultivar showed no differences length of stomata in outer epidermis (i.e. 26.488±1.288 µm and 26.488±2.962 µm). In triploid level group of banana, the length of stomata also showed no significantly different. The average of stomata length of Ambon warangan cultivar was of 33.572±2.284 µm, the average of Kluthuk susu cultivar's was 33.418±0.689 µm, and the average of Raja nangka cultivar's was 32.956±2.066 µm.

The highest number of stomata (mm^{-2}) in outer epidermis was found in Penjalin cultivar (AA genome): 14.55±7.476, with stomata index of 1.55%, while the lowest number was found in Kluthuk warangan (BB genome). The highest number of stomata in inner epidermis was found in Kluthuk warangan cultivar, and the lowest was found in Raja nangka. The density of stomata in outer epidermis layer was higher than that in inner epidermis layer. This condition was supposed to be related to the function of stomata. In outer epidermis layer, stomata were directly connected with atmosphere to catch oxygen, to facilitate their function as respiration, transpiration and photosynthesis processes. The anatomical characters of pseudostem of five cultivars were presented in Table 2 and Figure 3.

Table 2. The stomata characters of pseudostem and leaf in five banana cultivars (AA, BB, AAA, ABB, AAB) of different ploidy levels.

Character	Cultivar				
	Penjalin (AA)	Kluthuk warangan (BB)	Ambon warangan (AAA)	Kluthuk susu (ABB)	Raja nangka (AAB)
Characters of pseudostem stomata					
Outer stomata					
▪ Subsidiary cell	4 -7	4-6	4-6	4-8	4-6
▪ Length (µm)	26.488±1.288^{a}	26.488±2.962^{a}	33.572±2.284^{b}	33.418±0.689^{b}	32.956±2.066^{b}
▪ Width (µm)	26.488±2.755^{a}	34.804±2.066^{b}	31.416±4.016^{b}	42.50±2.335^{c}	41.58±4.747^{c}
▪ Number of stomata (per 1 mm²)	14.55±7.476^{b}	7.28±4.07^{a}	10.01±2.035^{ab}	9.1±3.217^{ab}	8.19±3.807^{ab}
▪ Number of epidermis (per 1 mm²)	924.56±75.89^{b}	1188.46±16.841^{d}	1090.18±0.133^{c}	987.35±100.636^{b}	839.02±46.133^{a}
▪ Stomata index	1.55%	0.61%	0.91%	0.91%	0.97%
Inner stomata					
▪ Subsidiary cell	4-6	4-5	4-6	4-5	4-5
▪ Length (µm)	27.412±2.284^{ab}	31.724±1.377^{bc}	35.42±3.772^{c}	28.644±5.51^{ab}	25.872±2.008^{a}
▪ Width(µm)	24.948±5.803^{a}	44.968±4.671^{b}	37.884±4.016^{b}	38.192±5.04^{b}	25.872±6.748^{a}
▪ Number of stomata (per 1 mm²)	7.28±4.07^{ab}	8.19±2.035^{b}	7.28±2.492^{ab}	5.46±2.035^{ab}	3.64±2.035^{a}
▪ Number of epidermis cells (per 1 mm²)	483.21±62.17^{d}	308.58±37.877^{b}	403.13±31.947^{c}	322.14±8.139^{b}	223.95±18.482^{a}
▪ Stomata index	1.48%	2.59%	1.77%	1.67%	1.61%
Character of leaf stomata					
Stomata of adaxial epidermis					
▪ Number of subsidiary cell	4-6	4	4-5	4	4-5
▪ Length (µm)	27.412±1.687^{a}	26.488±2.530^{a}	33.88±1.089^{b}	32.032±1.756^{b}	35.112±2.008^{b}
▪ Width (µm)	28.028±1.756^{a}	27.412±2.755^{a}	30.492±1.687^{ab}	34.804±2.577^{c}	32.956±1.377^{bc}
▪ Number of epidermis (per 1 mm²)	1716.26±139.144^{b}	1509.69±293.209^{b}	2063.88±180.933^{c}	1708.98±297.436^{b}	975.52±104.778^{a}
▪ Number of stomata (per 1 mm²)	49.14±4.984^{d}	13.65±4.55^{a}	37.31±9.864^{c}	16.38±7.614^{ab}	22.75±3.217^{b}
▪ Stomata index	2.78%	0.90%	1.78%	0.95%	2.28%
Stomata of abaxial epidermis					
▪ Number of subsidiary cell	4	4	4	4	4
▪ Length (µm)	23.1±1,54^{a}	24.024±1.756^{ab}	29.568±1.288^{c}	26.796±2.577^{bc}	24.948±3.339^{ab}
▪ Width (µm)	27.104±3.002^{bc}	30.184±1.756^{c}	24.024±1.756^{b}	24.332±2.284^{b}	19.866±3.285^{a}
▪ Epidermis number (per 1mm²)	1076.53±108.658^{c}	594.23±72.771^{ab}	1138.41±112.072^{c}	515.06±76.434^{a}	715.26±101.302^{b}
▪ Stomata number (per 1 mm²)	141.05±12.461^{b}	192.01±18.870^{c}	125.58±6.900^{a}	190.19±3.807^{c}	125.58±4.07^{a}
▪ Stomata Index	11.58%	24.42%	9.94%	26.97%	14.94%

Note: values followed by different rates in the same column are not significantly different in DMRT with α = 5%.

Morphology and anatomy of leaf

Morphology

The leaf of banana belongs to the complete type, because they have midrib, stalk (petiole) and blade (lamina). In banana, modification of midrib was called pseudostem, surrounding the true stem. In cross section the shape of petiole was look-like half circle and the dorsal side was shallow grooved or deep grooved. The side part of Penjalin (AA genome) petiole and Ambon warangan (AAA genome) cultivar boarded and look-like wing. Two other cultivars (BB and ABB genome) petiole their side part were close, while Raja nangka cultivar (AAB genome) as hybrid from natural crossing between *M. acuminata* (AA genome) and *M. balbisiana* (BB) the side part of petiole was open upright. According to Jumari (2007) AAB genome was originated from two set genome of *M. acuminata* (AA) and one set genome of *M. balbisiana* (BB). The shape of the five cultivars leaves is oblong with flat tip and entire margin. The ratio of the length and the wide of *M. paradisiaca* leaf was (2.5 -5): 1. According to Nugroho et al. (2003), this condition was oblong type.

Anatomy

Petiole composed of three tissue systems i.e.: epidermis layer, ground tissue system (parenchyma tissue) and vascular system. Morphological and anatomical examination of the petioles and leaves of *Musa textilis* suggested how these two apparently incompatible abilities are achieved. The hollow U-shaped section of the petiole and the longitudinal strengthening elements in its outer skin give it adequate rigidity, while its ventral curvature help support the leaf without the need for thick lateral veins. These features, however, also allow the petiole to reconfigure by twisting away from the wind, while the leaf can fold away. In addition, two sets of internal structures, longitudinal partitions and transverse stellate parenchyma plates, help prevent dorsoventral flattening, allowing the petiole to flex further away from the wind without buckling (Ennos et al. 2000).

Epidermis layer composed of a single layer, with compact cell, rectangular shape and was protected by cuticle. The position of parenchyma cells was irregular. In the middle part of the petiole, there were big air space and the parenchyma cells filled with air. The shape of air parenchyma cells was star-like, and formed network to each other. Many crystal needle-like and laticifer were distributed between those cells. In *Rustia formosa* (Rubiaceae), both the adaxial and abaxial epidermis are composed of polygonal cells. The epidermis cells in maize leaf were uniformly. The juvenile adaxial leaves of maize were covered with epicuticular wax, lack of hair and bulliform cell, whereas the adult leaf is pubescent with

bulliform cells but lack epicuticular waxes. In contrast, the adaxial epidermis in rice and bluegrasses leaf was covered with both epicuticular waxes and hairs (Sylvester et al. 2001).

Vascular bundles in petiole consisted of two groups, first group small located beneath the epidermis layer row regularly, while the big one distributed irregularly in the inner side. The type of vascular bundle was close collateral, consisted of xylem and phloem elements, and both were surrounded by thick wall (sclerenchyma). In big vascular bundle, the xylem consisted of vessel and tracheid, while the small one consisted of vessel only. The position of xylem in petiole was in upper side while phloem in lower side. Anatomy of petiole presented in Figure 4.

Blade (lamina). The blade consisted of epidermis layer, vascular bundles and parenchyma cells. The shape of epidermis cells was rectangular. The longest epidermis size was found in Penjalin cultivar, the widest was found in Kluthuk susu cultivar, the shortest was found in Raja nangka cultivar, and the narrowest one was found in Kluthuk warangan. The size of adaxial epidermis was bigger than that of abaxial epidermis. In Penjalin and Kluthuk susu cultivars hypodermis layer was found beneath the upper epidermis layer, whereas Kluthuk susu cultivar had two layers of hypodermis. Hypodermis were not found in the other three banana cultivars. According to Vieira et al. (2001) research, both the adaxial and abaxial epidermis of. *R. formosa* are composed of polygonal cells. Similar with the Kluthuk susu cultivar, the adaxial epidermis of this species were composed of two layer of cells, while the abaxial one was a single layer.

The abaxial epidermis of banana leaf was covered with cuticle. Trichome (hair) was not found in epidermis layer of banana. Epidermis, hair layer and cuticle as protective tissues that first intercept radiation. This tissues protecting the leaves against ultraviolet-B radiation (Karabourniotis et al. 1998).

Mesophyll (tissue between adaxial and abaxial epidermis) consisted of palisade and spongy tissues. These tissues consist of chloroplast which contains chlorophyll pigment. There were two palisade layers were found and has dense arrangement. Some of spongy cells was breakdown and formed big airspace. Some of spongy cells filled with few chloroplasts. This condition is the general structure of banana leaf (Tomlison 1969). The size of airspace and the thick of mesophyll of five cultivars showed different one to the other.

Similar with banana, mesophyll of *R. formosa* also composed of two palisade and several layer of spongy parenchyma, but no airspace were found (Vieira et al. 2001).The typical of both leaf based on the mesophyll composition was dorsiventral. Spongy parenchyma consists of thin-walled cell, and irregularly placed. The airspace in spongy layer may be lysigenous or schizogenous origin. Research of Turner (1999) and Turner et al. (1998) showed that most cavities and canals in leaf mesophyll thought to be lysigenous and schizogenous origin. Turner et al. (1998) presented evidence that lysigenous appearance in *Citrus lemon*, and schizogenous origin was found in *R. formosa* (Vieira et al. 2001)

Vascular tissues distributed in mesophyll, consisted of small and big. The big vascular bundle composed of vessel, tracheid, fiber, parenchyma cells and phloem (Tomlison 1969). The vascular bundle of the five cultivars is composed of xylem and phloem elements. The bundle surrounded by the parenchymatic or sclerenchymatic cells, was called bundle sheath. The small bundles were not protected by bundle sheath. Laticifer were scattered between palisade cells or in spongy tissue near the abaxial epidermis. Raja nangka cultivar produced fewest laticifer. The anatomy character of leaf blade can be seen Figure 5.

*Stomata***.** Stomata were found on both surface of epidermis layer. The type of stomata was phanerophor because the position of guard cell in line with epidermis layer. This result was supposed by Tomlison's research (1969). The shape of guard cell was kidney-like. Each stoma was surrounded by 4-6 cells. The distribution, the size and the index of stomata were varied in five banana cultivars. The size of triploid stomata on the upper and lower epidermis layer of leaves longer than the diploid one. The ploidy level affects this character significantly to the length and the width of stomata in upper epidermis layer. The length of stomata in diploid cultivars has no significantly different, as well as in triploid cultivars. In abaxial epidermis of Kluthuk warangan distribution of stomata was higher than the others (192.01±18.87) (see Table 2.).The number of subsidiary cells was four to six cells. The number of subsidiary cells in *R. formosa* three to six with various shape (Vieira et al. 2001). Stomata present only in the abaxial surface with the calculated average number of 133 stomata/mm^2. The type of stomata was predominantly paracytic. The size and distribution of banana stomata were presented in Table 2.

CONCLUSION

Stem and leaf morphology character of diploid level (AA and BB genome) was different from triploid level (AAA, AAB, and ABB genome). Anatomy and morphology character of root and rhizome of banana in diploid level and triploid level was quite similar. Distribution of stomata was found in leaf and pseudostem. Stomata were found in adaxial and abaxial epidermis layer. The size of guard cells in triploid cultivars is longer than that diploid cultivars. The root composes of epidermis layer, cortex and cylinder vascular of five cultivar's root show anomalous structure. Rhizome consists of peripheric and centre zone. Anatomically, there was no difference in the rhizome structure between five banana cultivars. The row of vascular bundles acts as demarcation area between periphery and central zone. In cultivar with BB genome (diploid) and ABB genome (triploid) the row of vascular bundle was not found. The differences of leaf anatomy are base on: size and number of stomata distribution, number of subsidiary cells, number of hypodermal layer, structure and number of parenchyma palisade, size of airspace in petiole and mesophyll and the vascular bundle structure.

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