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Pollen Morphology of Native Banana Cultivar (*Musa acuminata* Colla) in Surat Thani Province

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Abstract—A pollen morphological study of banana cultivar (*Musa acuminata* Colla) in Surat Thani Province conducted from March 2015 to February 2016. The pollen size and exine sculpturing of 8 cultivars of banana were studied by light and field emission scanning electron microscopy. The 8 pollen samples were acetolyzed following the technique of Erdtman (1969). The pollen is spheroidal. In addition, the aperture types are colporate. The numbers of aperture found were one aperture. The exine sculpturing is psilate. The length of pollen grains in average was 45-122 μ m. Pollen size of banana cultivar genome B was larger than of genome A whereas triploid banana cultivar could be produced larger pollen size compared to diploid. The pollen size is differing among the banana cultivars. Therefore, the pollen morphology could be usefully identified banana cultivars.

Index Terms—Musa, pollen, scanning electron microscope

I. INTRODUCTION

Banana is an important food crop of Musaceae family distributes in South East Asia, Australia and Africa. Therefore, it is cheap and has been grown in most parts of Thailand. It contains carbohydrate, chemical composition, minerals and vitamins [1]. The cultivated banana is a predominantly triploids and develops fruits by vegetative parthenocarpy. Although, wild banana, plantain and banana breeding has attempted to mimic the evolutionary development of the *Musa* species complex from inter-specific hybridization and polyploidization involving *Musa* sp. Moreover, there is a lack of study looking at the knowledge of pollen morphology of native banana, especially Thai banana varieties. The objective of this study was to evaluate the pollen morphology of native banana cultivar and to determine the floral structure of banana cultivar from Surat Thani Province, Thailand.

The male flowers of banana are around 6 cm. long with 5 stamens, showing 2 whorls, with one stamen in adaxial position, opposite free tepal missing anther long 2 cm., basifixed, introrse, good developed, but rarely holding pollen in the cultivars: too small unsuccessful ovary, about one-four part of the length of banana flower with plump style and stigma, entire flowers cut off at base of unsuccessful ovary [1]. After that, male and female of banana flowers can make abundant nectar and pollen is having the property of adhering, as glue, adhesive at maturity, consequently, proposing that biotic pollination

in banana is insect pollination is a form of pollination whereby pollen of pollen banana and animal based. Therefore, the banana flowers are normal visited by bats, rats, birds, ants, bees, wasps and other large insects [1].

The *Musa* sp. were classified from *Ensete* sp. based study on for example, study from; Kress, 1990 showed three genera of the Musaceae, L. (Linnaeus, 1753) reported about 65 species *Ensete* Bruce ex Horan (Horanineu, 1862) reported 7 species *Musella* sp. (Franch) H.W.Li (Li, 1978) about one specie banana in Thailand Simmonds 1956, Smithinand 2001 native species in Thailand *Musa acuminata* Colla, *M. gracilis* Holttum, *M. itinerans* Cheesman, *M. laterita* Cheesman and *M. balbisiana* Colla Swangpolet *et al.* 2007 and Swangpol and Somana 2009 reported banana species introduced as ornamental plants example *M. velutina* Wendl. & Drude, *M. coccinea* Andr. and *M. ornate* Roxb. [2]

Working on pollen morphology, viability and the relationship between the pollen size and banana cultivars is very creative and important for biology and botany, Nicol *et al.* (2017) [3] and Ni *et al.* (2018) [4] used LM and FESEM (field emission scanning electron microscopes)

According to Zarrel *et al.* (2010) [5] anatomical pattern is an important taxonomic characteristic in many plant groups, expected in monocot. However, in part of the anatomy of Musaceae of two vegetative and reproductive units example leaves, shoot, rhizome and flower have been commonly reported [5]-[8]. Pollen morphology of Musaceae species has not been studied. The aim of this study was, therefore, to describe the pollen analysis of Musaceae found in Surat Thani, Thailand in order to help the identification.

II. METHODOLOGY

Eight banana cultivars including *Musa ornate* (Kluai-buchompu), *M. acuminata* AA (Kluai-kai), *M. acuminata* AA (Kluai-lepmunai), *M. acuminata* AA (Kluai-par), *M. acuminata* BB (Kluai-tanee), *M. acuminata* AAA (Kluai-homchaiya), *M. acuminata* ABB (Kluai-mumvasikao), and *M. acuminata* BBA (Kluai-mumvasilung) were collected during March 2015 to February 2016 in Surat Thani Province. 20 pollen grains of each banana species were taken from fresh flowers obtained from a garden at Surat Thani Province. All samples were prepared under uniform conditions at both the Department of Biology, Faculty of Science and

Technology, Suratthani Rajabhat University, and the Science Center, the Center for Scientific and Technological Equipment, Walailak University, so that the pollen grains could be examined under both light and field emission scanning electron microscopes (LM and FESEM), respectively.

For FESEM, the samples were first chemically processed according to a method developed by Erdtman (1969) [9]. Each sample was immersed in an acetolysis solution. Banana pollen specimens were dehydrated in an ethanol series and mounted on a stub with adhesive carbon tape, sputter coated with gold, and examined under a FESEM. All pollen grains were observed and photographed in polar and equatorial views at a magnification of x400 and x1,000, using a Nikon Eclipse E600 LM equipped with a Canon EOS 1100D digital camera and using a Zeiss model Merlin compact Gemini Field Emission Scanning Electron Microscope (FESEM) On average, 20 pollen grains of each species were used and measured.

III. RESULTS

The pollen morphology of eight native banana cultivar (*Musa acuminata* Colla) found in Surat Thani Province was collected including *Musa ornate* (Kluai-buchompu) as shown in Figure 1, *M. acuminata* AA (Kluai-kai), *M. acuminata* AA (Kluai-lepmunai), *M. acuminata* AA (Kluai-par), *M. acuminata* BB (Kluai-tanee), *M. acuminata* AAA (Kluai-homchaiya) as shown in Figure 2 left, *M. acuminata* ABB (Kluai-mumvasikao) as shown in Figure 2 right, and *M. acuminata* BBA (Kluai-mumvasilung). In Surat Thani, Kluai-buchompu *Musa ornate* is a rare species and normally used for ornamental plant, whereas Kluai-homchaiya and Kluai-mumvasikao are found and consumed locally.

The material from eight populations of eight species of native banana cultivar were collected and studied from the field in Surat Thani Province as indicated in Table I, and Pollen morphology of banana cultivar (*Musa*

acuminata Colla) in Surat Thani Province can be classified into 8 pollen types based on pollen shapes and sizes as showed in Table II and Table III.



Figure 1. Kluai-buchompu (*Musa ornate* Roxb).



Figure 2. Kluai-homchaiy (*Musa acuminata* AAA) (left); Kluai-mumvasikao (*M. acuminata* AAB) (right).

TABLE I. LIST OF *MUSA* SP. MATERIALS USED IN THIS STUDY SPECIMENS IN SURAT THANI PROVINCE, THAILAND

Species	Vouchers	Locality
<i>Musa ornata</i>	P.K. 101	Amphermuang, Muang District
<i>M. acuminata</i> AA	P.K. 102	Subtawee, Bangnaduam District
<i>M. acuminata</i> AA	P.K. 103	Lipanoi, KohSamui District
<i>M. acuminata</i> AA	P.K. 104	Bangsadat, Kangsa District
<i>M. acuminata</i> BB	P.K. 105	Thakanon, Kiriratnikom District
<i>M. acuminata</i> AAA	P.K. 106	Lamet, Chaiya District
<i>M. acuminata</i> ABB	P.K. 107	Lelet, Phumphin District
<i>M. acuminata</i> BBA	P.K. 108	Krud, Kanchanadit District

TABLE II. A SUMMARY OF THE POLLEN MORPHOLOGICAL CHARACTERISTICS AND POLAR AXIS AND AN EQUATORIAL AXIS OF NATIVE BANANA CULTIVAR POLLEN EXAMINED (MICRON)

Scientific name	Thai name	Pollen type	Type of aperture	Number of aperture	Polar axis (micron)	Equatorial axis (micron)	Exine thickness (micron)
<i>Musa acuminata</i>	Kluai-par	spheroidal	Colporate	1	50-65	52-66	1
<i>Musa balbisiana</i>	Kluai-tanee	spheroidal	Colporate	1	80-85	82-86	1
<i>Musa ornata</i>	Kluai-buchompu	spheroidal	Colporate	1	45-55	45-53	1
<i>Musa acuminata</i> AA	Kluai-kai	spheroidal	Colporate	1	50-55	52-56	1
<i>Musa acuminata</i> AA	Kluai-lepmunai	spheroidal	Colporate	1	52-55	52-54	1
<i>Musa acuminata</i> AAA	Kluai-homchaiya	spheroidal	Colporate	1	90-95	92-96	1
<i>Musa acuminata</i> ABB	Kluai-munvasaikao	spheroidal	Colporate	1	100-104	102-105	1-2
<i>Musa acuminata</i> BBA	Kluai-munvasilung	spheroidal	Colporate	1	110-114	112-114	1-2

TABLE III. A SUMMARY OF THE POLLEN MORPHOLOGICAL CHARACTERISTICS AND SIZE MEASUREMENTS OF NATIVE BANANA CULTIVAR POLLEN EXAMINED (MICRON)

Scientific name	Thai name	Pollen size (micron)	mean±SD
<i>Musa ornata</i>	Kluai-buchompu	40-53	44.31±4.52
<i>Musa acuminata</i> AA	Kluai-kai	42-56	47.24±3.48
<i>Musa acuminata</i> AA	Kluai-lepmumai	52-54	53.65±1.65
<i>Musa acuminata</i> AA	Kluai-par	52-66	58.83±3.61
<i>Musa balbisiana</i> BB	Kluai-tanee	70-80	75.54±5.35
<i>Musa acuminata</i> AAA	Kluai-homchaiya	92-96	94.57±2.74
<i>Musa acuminata</i> ABB	Kluai-mumvasikao	102-105	103.63±1.84
<i>Musa acuminata</i> BBA	Kluai-mumvasilung	112-114	113.76±1.49

It is clear from Table II that each pollen morphological characteristics of eight banana cultivars including *Musa ornate* (Kluai-buchompu), *M. acuminata* AA (Kluai-kai), *M. acuminata* AA (Kluai-lepmunai), *M. acuminata* AA (Kluai-par), *M. acuminata* BB (Kluai-tanee), *M. acuminata* AAA (Kluai-homchaiya), *M. acuminata* ABB (Kluai-mumvasikao), and *Musa acuminata* BBA (Kluai-mumvasilung) is all spheroidal, the type of aperture is colpate, number of aperture is one are identified. Next, it is clear from Table III that the length of pollen grains in average was 45-122 μm. The biggest of pollen size is *M. acuminata* BBA (Kluai-mumvasilung) was 113.76±1.49 micron and the smallest of pollen size is *Musa ornate* (Kluai-buchompu) was 44.31±4.52 micron.

The description of pollen morphological characteristic can be described in text below and their photograph which taken from light microscope and scanning electron microscope as shown in Figure 3.

Musa acuminata sub.sp. *malaccensis* AA (Fig. 3.1A-1B):

Monads, radially symmetrical, isopolar, P = 50-65 micron, E = 52-66 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa balbisiana BB (Fig. 3.2A-2B):

Monads, radially symmetrical, isopolar, P = 80-85 micron, E = 82-86 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa ornata (Fig. 3.3A-3B):

Monads, radially symmetrical, isopolar, P = 45-53 micron, E = 45-53 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa acuminata AA (Fig.3.4A-4B):

Monads, radially symmetrical, isopolar, P = 50-55 micron, E = 52-56 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa acuminata AA (Fig. 3.5A-5B):

Monads, radially symmetrical, isopolar, P = 52-55 micron, E = 52-54 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa acuminata AAA (Fig. 3.6A-6B):

Monads, radially symmetrical, isopolar, P = 90-95 micron, E = 92-96 micron, small, spheroidal, colpate, one aperture, exine thickness 1 micron, psilate
Musa acuminata ABB (Fig. 3.7A-7B):

Monads, radially symmetrical, isopolar, P = 100-104 micron, E = 102-105 micron, large, spheroidal, colpate, one aperture, exine thickness 1-2 micron, psilate
Musa acuminata BBA (Fig. 3.8A-8B):

Monads, radially symmetrical, isopolar, P = 110-114 micron, E = 112-114 micron, large, spheroidal, colpate, one aperture, exine thickness 1-2 micron, psilate

Moreover, the study showed that the pollen morphological characteristics of banana (Musaceae) are same uniform but pollen size varied among banana cultivar that can be used to make key pollen type.

Key to pollen type

1. Pollen size less than 45 micron Kluai-buchompu
1. Pollen size more than 45 micron 2
2. Pollen size 45-50 micron Kluai-kai
2. Pollen size more than 50 micron 3
3. Pollen size 50-52 micron Kluai-lepmumai
3. Pollen size more than 52 micron 4
4. Pollen size 52-60 micron Kluai-par
4. Pollen size more than 60 micron 5
5. Pollen size 60-80 micron Kluai-tanee
5. Pollen size more than 80 micron 6
6. Pollen size 90-100 micron Kluai-homchaiya
6. Pollen size more than 100 micron 7
7. Pollen size 100-110 micron Kluai-mumvasikao
7. Pollen size more than 110 micron Kluai-mumvasilung

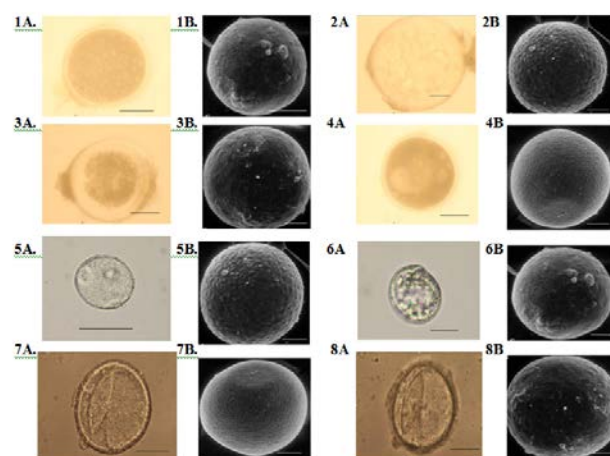


Figure 3. 1: Pollen of *Musa acuminata* Colla sub sp. *malaccensis* (Ridl) Simmonds (A) from LM x400; (B) from FESEM scale 25 micron. 2: Pollen of *M. balbisiana* Colla (A) from LM x400; (B) from FESEM scale 25 micron. 3: Pollen of *M. ornate* Roxb (A) from LM x400; (B) from FESEM scale 25 micron. 4: Pollen of *M. acuminata* Colla AA (A) from LM x400; (B) from FESEM scale 25 micron. 5: Pollen of *M. acuminata* Colla AA (A) from LM x400; (B) from FESEM scale 25 micron. 6: Pollen of *M. acuminata* Colla AAA (A) from LM x400; (B) from FESEM scale 25 micron. 7: Pollen of *M. acuminata* Colla ABB (A) from LM x400; (B) from FESEM scale 25 micron. 8: Pollen of *M. acuminata* Colla BBA (A) from LM x400; (B) from FESEM scale 25 micron.

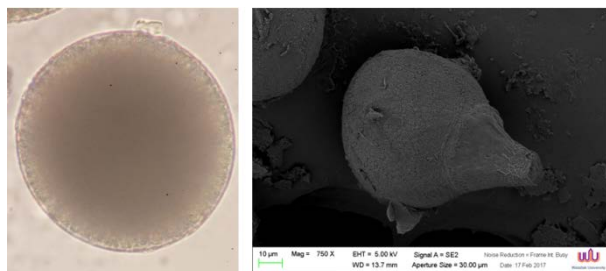


Figure 4. Left is Pollen of Kluai-par (*M. acuminata* AA) from LM x100. Right is germination of (*M. acuminata* AA) from FESEM x750.

The viability and germination of Kluai-par (*M. acuminata* AA) was observed under Light Microscope. The results are shown in Figure 4. The percent of pollen viability reduced in the time of storage. At 0°C and 10-15°C the viability was higher than that at the room temperature (25°C)

IV. DISCUSSIONS AND CONCLUSIONS

A total of 8 cultivars of banana can found in Suratthani Province. After examine following the technique of Erdtman [9], the pollens of all 8 banana cultivar are spheroidal. In addition, the aperture types are all colporate and the numbers of aperture found were one aperture. The exine sculpturing is psilate. In term of pollen morphology, the result of this study (*Musa ornanta* and *M. acuminata*) is in line with the study of Wandee et al. [10], determined flora micro morphology of genus *Ensete* Bruce ex Horan (Musaceae) in Thailand. However, the pollen size is differing among the banana cultivars. In term of pollen viability and pollen germination of the Kluai-par (*M. acuminata* AA) the result of this study is similar to Silayoi (1990) [11]. The length of pollen grains in average was 45-122 μm. Pollen size of banana cultivar genome B was larger than of genome A whereas triploid banana cultivar could be produced larger pollen size compared to diploid.[10]-[13]. Therefore, the result regarding the pollen morphology found in this study could be useful to identify banana cultivars.

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