Systematic Significance of Leaf Anatomy in *Johannesteijsmannia* H.E. Moore (Arecaceae)

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ABSTRACT

A comparative leaf anatomy study was undertaken on *Johannesteijsmannia* H.E. Moore in Peninsular Malaysia in order to assess leaf anatomical variation which may be useful in species identification. All four species representatives of Malaysian Johannesteijsmannia were studied. Idioblast cells, sclereids, sclerenchymatous inner bundle sheaths, parenchymatous outer bundle sheaths, division of phloem in the vascular bundles, sub-epidermal fibrous strands below abaxial and adaxial epidermis are commonly present in all the species studied. The outline of the leaf margins, types of stomata, shaped of epidermis cells on the adaxial side, the position of vascular bundles in the leaf lamina and the presence of simple unicellular trichome, fibres at the tip of leaf margins, sub-epidermal fibrous strands in the mesophylls cells, hypodermis layer below adaxial epidermis and stegmata are useful in distinguishing individual species. In conclusion, anatomical characters have systematic significance in Johannesteijsmannia, especially at the species level.

Keywords: Systematic, leaf anatomy, Johannesteijsmannia, palms

INTRODUCTION

Johannesteijsmannia H.E. Moore is a genus in the tribe Corypheae Mart., subfamily Coryphoideae Griff. (Arecaceae), found in the tropical rainforests of southern Thailand, Malaysia and Indonesia (Uhl & Dransfield, 1987). The palms with huge, diamond-shaped fronds, known as daun payong, umbrella leaves, sal, sand, koh, lak, are one of the grand sights of the Malaysian forest (Lim & Whitmore, 2000; Tomlinson, 1990). Most of the Johannesteijsmannia species, especially J. magnifica and J. altifrons, are widely used in landscapes and popular as ornamental plants because of the uniqueness of their architecture. There are four species distributed within the Peninsula, of which the most widespread is J. altifrons (Rchb. f. and Zoll.) H. E. Moore, that is also native to south Thailand, north Sumatra, and Sarawak (Lim & Whitmore, 2000). The other three, J. magnifica J. Dransf., J. lanceolata J. Dransf. and J. perakensis J. Dransf., are endemic to Peninsular Malaysia, and are relatively rare and highly endangered (Dransfield, 1972). The four species of the genus were previously assumed to be the same, originally named Teysmannia altifrons, discovered c. 1856 in Padang, Sumatra, by K.F. Stijman who sent it to J.E. Teysmann (also spelt Teijsmann). It was formally published in 1853 by Reichenbach and Zollinger, but in 1961, H.E. Moore Jr. changed the genus name to Johannesteijmannia H.E. Moore (Moore, 1961).

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The first attempt to study the anatomy of palm was by Solereder and Meyer (1928), however, the work suffered poor illustration. The most comprehensive study was conducted on T. altifrons by Tomlinson (1961). This article provides a guide for the leaf anatomical data on the four species of Johannesteijsmannia in detail. The overall objective of this study was to determine whether the leaf anatomical characters in Johannesteijsmannia could be of taxonomic value in systematic and diagnostic investigations, besides providing additional data on the leaf anatomical characters of this special genus. These anatomical characteristics would provide supporting evidence for the systematic treatment of the genus Johannesteijsmannia.

MATERIALS AND METHODS

Fresh specimens used in this study were obtained from various localities in Peninsular Malaysia, including the palm collection of Forest Research Institute, Malaysia, Kepong, Selangor, Gunung Angsi Forest Reserve, Negeri Sembilan and Kledang Sayong Forest Reserve, Perak, Gunung Bubu Forest Reserve, Perak and Semenyih Dam, Selangor. Three replicates were used to represent each species in this study. Voucher specimens of the freshly collected material were prepared by dry pressing, followed by mounting them onto the herbarium sheet, labelling and documenting. The voucher specimens were kept at Universiti Kebangsaan Malaysia Herbarium, Malaysia (UKMB) for future reference. A full list of the species studied and the localities from which they had been collected is given in Appendix 1. Fixation, embedding, sectioning, epidermal mechanical scrapping and stained were done according to the procedure by Johansen (1940) and Sass (1958), with suitable modifications. Fresh materials were fixed in AA (1:3) of acetic acid (30%) acetic acid and ethanol (70%). Dried herbarium materials were boiled, and fixed using the same solution. Leaf transverse section of the specimens were sectioned with a sliding microtome at $20 - 30 \mu m$ thickness and stained in 1 % Safranin in 50 % alcohol and 1 % Alcian Green in 100 ml purified water with three drops

of acetic acid. Sections of the leaf marginal and lamina were made from the middle and marginal parts of the leaf lamina using a Reichert sliding microtome. All slides were mounted in Euparal after dehydration using alcohol series 50%, 70%, 95% and 100%. Photomicrographs of the sections were taken using either a Leitz Diaplan polarizing microscope fitted with a JVC CCD camera or Reichert Polyvar 2 Microscope fitted with a digital camera. These images were processed using Analysis Docu Software (softimaging system). All the slides were deposited in the anatomy section at Microtechnique Laboratory, Universiti Kebangsaan Malaysia, Malaysia.

RESULTS

Results showed some variations and similarities in the leaf anatomical characteristics of *Johannesteijsmannia* species studied. A summary of the leaf anatomical characteristics observed in this study is presented in Tables 1 and 2.

Epidermal cells anticlinal and periclinal walls: straight to curved anticlinal walls on the abaxial and adaxial epidermis of J. altifrons, J. lanceolata, J. magnifica and J. perakensis (Fig. 1A - H). Stomatal complexes: hypostomatic (present only on the abaxial epidermis); hexacytic in J. altifrons (Fig. 2A) and J. perakensis (Fig. 2D), tetracytic in J. lanceolata (Fig. 2B), octacytic in J. magnifica (Fig. 2C). Trichomes: present only on the abaxial epidermis of J. magnifica, simple multicellular trichomes (Fig. 4M). Shaped of epidermal cells: rectangular in J. altifrons (Fig. 1E), J. lanceolata (Fig. 1F) and J. perakensis (Fig. 1H), mostly hexagonal in J. magnifica (Fig. 1G). Margin outline: rounded in J. magnifica (Fig. 4C), pointed in J. altifrons (Fig. 4A), J. lanceolata (Fig. 4B) and J. perakensis (Fig. 4D). Meanwhile, fibrous cells were found to be present at the tip of the leaf margin of J. lanceolata (Fig. 4B), J. magnifica (Fig. 4C) and J. perakensis (Fig. 4D), but absent in J. altifrons (Fig. 4A). The presence of secondary and tertiary vascular bundles varies between the species; these are sparsely scattered

in J. altifrons and J. lanceolata, but densely scattered in J. magnifica and J. perakensis. Leaf lamina: adaxial and abaxial epidermal cells, 1 layer in J. altifrons (Fig. 3A), J. lanceolata (Fig. 3B), J. perakensis (Fig. 3D) and in J. magnifica (*Fig. 3C*). Hypodermis: one layer under adaxial epidermal cells, interrupted by sclerenchyma cells or fibrous strands or sub-epidermal fibres. was shown to be present in J. altifrons (Fig. 31) and J. magnifica (Fig. 3C), and one layer of uniform hypodermis cells under adaxial epidermal cells in J. lanceolata (Fig. 3B) and J. perakensis (Fig. 3D). Chlorenchyma: palisade cells in 2 to 3 adaxial layers occupying 1/3 of leaf thickness, one cell to three times taller than the width. Spongy mesophyll cells in ca 6 - 8 layers are round, with no intercellular spaces. Vascular bundles in the leaf lamina: arranged in one row, approximately equidistant from the abaxial and adaxial epidermal layers in J. lanceolata (Fig. 3B) and J. perakensis (Fig. 3D), whereas these are close to abaxial epidermis in J. altifrons (Fig. 3A) and J. magnifica (Fig. 3C). Bundle sheaths, inner sheath; sclerenchyma cells completely ensheathing the vascular bundles, outer sheathparenchyma cells in one layer incompletely ensheathing the vascular bundles (*Fig.* 3A - H). Phloem was divided into two sections by the layers of sclerenchyma cells that were present in all the species studied. Sclerenchyma cells: clusters of sclerenchyma cells or sub-epidermal fibres present under the adaxial and abaxial epidermis in all the species studied (*Fig.* 3A - L). Sub-epidermal fibres were also present between the mesophyll cells in J. altifrons (Fig. 3A), J. lanceolata (Fig. 3B) and J. perakensis (Fig. 3D), but absent in J. magnifica (Fig. 3C). Cell inclusions: silica bodies or stegmata alongside or close to the sub-epidermal fibres were present in J. lanceolata (Fig. 5A) and J. perakensis, but absent in J. magnifica and J. altifrons; idioblast cells present in the mesophyll cells very sparsely scattered in all the species studied, J. altifrons (Fig. 5C and 5I); sclereids were found in spongy mesophyll or associated with fibrous vascular bundle sheaths in all the species studied, namely J. magnifica (Fig. 5B), J. altifrons (Fig. 5C), J. perakensis (Fig. 5D) and J. lanceolata (Fig. 5E).

Trichomes: simple unicellular were shown to be very sparsely scattered and present only on the abaxial epidermis of *J. magnifica* (*Fig. 4K*) but absent in *J. altifrons, J. lanceolata* and *J. perakensis.*

DISCUSSION AND TAXONOMIC IMPLICATIONS

Lamina Transverse Sections

Tomlinson (1961) reported the presence of onelayered hypodermal cells below the abaxial and adaxial epidermal cells in some Arecaceae species. Cell files, which are often replaced by fibres and cells, are normally small, more or less cubical, and slightly thick-walled. The hypodermis layer is present in all the four species studied. In J. altifrons and J. magnifica, they are similar with one-layered below the adaxial epidermal cells, but interrupted with nonvascular fibres. It appears that J. lanceolata and J. perakensis have a uniform hypodermis layer. The cells are more or less cubical, slightly thick walled with two or three small sclerotic cells surrounding each sub-stomatal chamber. In the previous study in Licuala, one layer of hypodermal cells below the abaxial and adaxial epidermal cells were observed (Tomlinson, 1961). Adaxial cells are more or less cubical, most conspicuous and uniform. Hypodermal cells present in the abaxial epidermal cells are less regular and smaller. The result of this study has shown that the presence of hypodermis layer can be a diagnostic character for Johannesteijsmannia, and thus, it has taxonomic significance.

Veins are rather remote from each other, more or less equidistant and always separated from the epidermis by spongy and palisade mesophyll. The position of vascular bundles in leaf lamina is approximately equidistant from the abaxial and adaxial epidermal layers in the two species (*J. lanceolata* and *J. perakensis*), but close to abaxial epidermis in the other two spexies (*J. altifrons* and *J. magnifica*). All the *Johannesteijsmannia* species possess vascular bundles which are completely ensheathed

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TABLE 1

	Shaped of epidermis cells	Abaxial epidermis	Cell rectangular	Cell rectangular	Cell rectangular	Cell rectangular
		Adaxial epidermis	Cell rectangular	Cell rectangular	Cell hexagonal	Cell rectangular
	Anticlinal walls	Abaxial epidermis	Straight to curved	Straight to curved	Straight to curved	Straight to curved
		Adaxial epidermis	Straight to curved	Straight to curved	Straight to curved	Straight to curved
		Trichomes	Absent	Absent	Simple multicellular	Absent
	Stomata		Hexacytic	Tetracytic	Octacytic	Hexacytic
		Species	J. altifrons	J. lanceolata	J. magnifica	J. perakensis

TABLE 2 Leaf surface anatomical characteristics in *Johannesteijsmannia* species Systematic Significance of Leaf Anatomy in Johannesteijsmannia H.E. Moore (Arecaceae)

by sclerenchyma cells (as an inner sheath) and incompletely ensheathed by a layer of parenchyma cells (as an outer sheath). This study has shown that sclerenchyma cells that completely surrounded the vascular bundles and parenchyma cells that incompletely surrounded the vascular bundles in the lamina are common characters for all the Johannesteijsmannia species undertaken in the study. These characters are also common in most palms species (Tomlinson, 1961; Passos & De Mendonca, 2006), and therefore have less systematic value. The phloem tissues in the main vascular bundles of all the Johannesteijsmannia species studied have characteristics form with its phloem divided into two separating sections. This character is also found in some other palms species such as in Licuala rumphii, Mauritia flexuosa and Raphia vinifera (Tomlinson, 1961); therefore, this character also has less systematic value for distinguishing species in Johannesteijsmannia, yet taxonomically significant for the species in Areacaceae.

The outline of the transverse sections of the margin is pointed or rounded, pointed in J. altifrons, J. lanceolata and J. perakensis, but rounded in J. magnifica. Thus, it is certainly a diagnostic character for this particular species. The leaf margin is not frequently used in systematic study. However, Talip et al. (2005) stated that this character is useful in the identification of some Zingiberaceae species. This anatomical characteristic definitely has systematic significance in Johannesteijsmannia, especially in the presence of fibrous cells or strands at the tip margin. The fibrous cells were found to be present in J. lanceolata, J. magnifica and J. perakensis but absent in J. altifrons. The presence of marginal fibrous strands or cells in J. lanceolata, J. magnifica and J. perakensis has characteristic outlines. In this study, it was shown that J. lanceolata has marginal fibre strands shaped No. 3 ('V' shaped), while J. magnifica has marginal fibre strands shaped No. 1 (U shaped) and J. perakensis has marginal fibre strands shaped No. 2 (arc shaped). Wu and Cutler (1985) also stated that the presence of marginal fibrous strands is constant,

reliable diagnostically and taxonomically useful characters in Iris.

Cell Inclusions

Silica is most common in palms as small bodies and these silica cells are the stegmata named by German authors (Tomlinson, 1961). Stegmata are usually the most abundant adjacent to the vascular and fibrous bundles of the stem and leaf (Dickison, 2000). In this study, stegmata present in the mesophyll cells in long continuous files, adjacent to the abaxial fibrous strands in only two species, namely, *J. lanceolata* and *J. perakensis*. The silica bodies are spherical, which are rather irregular, more symmetrical and druse-like. The presence of stegmata in this study could therefore be very useful in distinguishing some species in this genus.

The presence of the sclereids and idioblast cells in the leaf lamina in all the species studied is a common anatomical character of this particular genus. Sclereids typically are short cells with thick secondary walls, strongly lignified and provided with numerous simple pits (Evert et al., 2006). Leaves are an especially rich source of sclereids; as for the variety of form, however, they are rare in the leaves of monocots (Tomlinson, 1959, 1961). The presence of sclereids is uncommon in palm leaves, apart from sclerotic mesophyll cells in some species in Eugeissona, Licuala, Daemonorops and Lodoicea (Tomlinson, 1959, 1961). The foliar sclereids found in Eugeissona are short, lignified and columnar, but long, unlignified and fibre-like in Bactris and Licuala (Tomlinson, 1959). In Daemonorops, fibre-like sclereids are present, and this can be homologous with the fibres ensheathing the tranverse veins. In this study, the foliar sclereids found to be present in mesophyll cells or attached to the vascular bundle sheaths. The sclereids were shown to be filiform, lignified and columnar, but long and present in all the Johannesteijsmannia species studied. Although sclereids in palms have no obvious biological function, they are quite useful in diagnosis especially at generic level.



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Fig. 1A-H: Leaf surface of epidermis in Johannesteijsmannia *species. Abaxial side:* A - D, (A) J. altiforns, (B) J. lanceolata, (C) J. magnifica, *and* D) J. perakensis. *Adaxial side:* E - H, (E) J. altifrons, (F) J. lanceolata, (G) J. magfinica, *and* (H) J. perakensis. *Scale bar 20 µm*



Fig. 2A - D: Types of stomata in Johannesteijsmannia *species; (A)* J. altifrons *(hexacytic), (B)* J. lanceolata, *(tetracytic), (C)* J. magnifica *(octacytic), and (D)* J. perakensis *(hexacytic). Scale bar: 20 μm*

Leaf Epidermis

Leaf epidermal characteristics are of taxonomical importance in many plants species (Stace, 1965, 1969; Wilkinson, 1979; Baranova, 1972, 1987, 1992a,b, 2004; Barthlott et al., 1998; Barthlott, 1981, 1990; Kong, 2001; Carpenter, 2005). The finding in this study has shown that this characteristic is of taxonomic value in the genus Johannesteijsmannia. The anticlinal wall of the adaxial and abaxial epidermal cells is straight to curvy in all the species studied, and appears to be not a good anatomical characteristic to differentiate the species. In the adaxial epidermal cells of J. altifrons, J. lanceolata and J. perakensis, the cuticular deposit has sinuous appearance, and this sinuous appearance of cuticular deposit occurs on the abaxial epidermal cells of all the species studied at a high level of focus, whilst at a low level, the wall is not sinuous. This characteristic was reported by Tomlinson (1961) in other palms genera. However, there are many palm species with sinuous anticlinal walls, such as in *Mauritia flexuosa* which can be a characteristic of this species within the genus (Passos & De Mendonca 2006).

In the abaxial epidermis above the hypodermal fibrous strands, there are files of long narrow cells (costal bands) which alternate with the files of wider cells (intercostals bands) elsewhere. Stomata are situated in the intercostals band and the files of the cells in the intercostals bands are quite similar between the species, and this is specifically 2 to 3 in *J. lanceolata* and *J. magnifica*, 2 to 4 in *J. perakensis* and with 3 to 5 files of the cells in *J. altifrons*. In the adaxial epidermal, there are also files of costal bands cells, cell rectangular,



Fig. 3A-L: Leaf lamina transverse sections; (A) J. altifrons, *(B)* J. lanceolata, *(C)* J. magnifica, *(D)* J. perakensis. *Vascular bundles; (E)* J. altifrons, *(F)* J. lanceolata, *(G)* J. magnifica, *(H)* J. perakensis, *(I)* J. altifrons, *an idioblast cell (black arrow) and an interrupted hypodermis layer (white arrow), (J)* J. lanceolata, *sub-epidermal fibrous cells (white arrow) and cutinized epidermal cells (black arrow), (K)* J. magnifica, *simple multicellular trichome, (L)* J. magnifica, *sub-epidermal fibrous cells below the abaxial epidermis (black arrow), and (M)* J. perakensis, *sub-epidermal fibrous cells below the abaxial epidermis. Scale bar: (A – D) = 100µm, E – I & K) = 50 µm, J, L & M) = 20 µm*

longitudinally extended, elongated parallel to the long axis of the leaf segment or leaflet, and some of the cells are almost cubical in all species studied. Nevertheless, the cells are hexagonal in shape in *J. magnifica*. The findings in this study have shown that the leaf epidermal characteristics can be used in distinguishing some species; therefore, this characteristic has its systematic significant in this genus.

Stomata

According to Solereder (1908), Metcalfe and Chalk (1950), Tomlinson (1961), Stace (1965) and Rajagopal (1979), the distribution patterns of stomata based on their orientation and dispersion are found to be fairly stable and could therefore be taxonomically useful. Stomata are restricted to abaxial intercostal regions and not in distinct or costal files of cells. The pattern of the stomata in this present study was classified as scattered and the stomata are often remote from each other. This result confirmed the observation made by Tomlinson (1961).

The common type of stomata in the palm species is paracytic (Tomlinson, 1961), or tetracytic (Tomlinson, 1961; Pérez & Rebollar, 2003). Tomlinson (1961) reported the type of stomata in this genus as being tetracytic and paracytic, while in this study, hexacytic stomata was found in *J. altifrons* and *J. perakensis*, tetracytic in *J. lanceolata* and octacytic in *J. magnifica*. In this study, it is therefore evident that the types of stomatal complexes have a significant taxonomic value in this genus, which can be used to differentiate between the four species.

CONCLUSIONS

Some of the anatomical characteristics observed in this study revealed a number of interesting



Fig. 4: Transverse sections of the leaf margins in Johannesteijsmannia species indicating margin shape and the presence of marginal fibre strands. (A) J. altifrons (pointed), (B) J. lanceolata (pointed), (C) J. magnifica (rounded), (D) J. perakensis (pointed). 1 = Marginal fibrous strands 'U' shaped (J. magnifica), 2 = Marginal fibrous strands arc shaped (J. perakensis), and 3 = Marginal fibrous strands 'V' shaped (J. lanceolata). Scale bar: (A- D) = 50 μm

features, with some characters which can be of taxonomic and diagnostic values. For example, sclereids and idioblast cells are common anatomical characters in the genus indicating close interrelationship between the species. The results of this study have shown that the leaf anatomical evidence can be used for identifying certain species in Johannesteijsmannia, such as in the presence of marginal fibre strands in all the species, except in J. altifrons, as the outline of the margin pointed in J. altifrons, J. lanceolata and J. perakensis but rounded in J. magnifica, Those characteristics provide some additional anatomical data of this particular genus. Meanwhile, the anatomical characters observed in this study revealed a number of interesting features with some characteristics which could be of taxonomic and diagnostic values. The features of the stomata may be diagnostic in J. lanceolata with tetracytic stomata and J. magnifica with octacytic stomata. Thus, the anatomical data obtained from the present study are useful to construct the artificial identification key to the species in Johannesteijsmannia.

Key to identification of four *Johannesteijsmannia* species using leaf anatomical characters:

- 2 Marginal fibrous strands 'V' shaped; stomata tetracytic..... J. lanceolata
- 2 Marginal fibrous strands arc shaped; stomata hexacytic......J. perakensis

Therefore, the anatomical features of this particular genus have been proven to be useful in the identification of species and they definitely have taxonomic values, specifically at the species level.

ACKNOWLEDGEMENTS

This study was financed by Universiti Kebangsaan Malaysia through research grant OUP-UKM-2009. The authors are grateful to Kamarudin Salleh for his help and guidance at the FRIM arboretum. Special thanks also go to Abu Hussin Harun for his technical assistance in the field.

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No	Species	Code	Locality	Collectors and date of collection
	J. altifrons	NT 100	FRIM Kepong, Selangor	Noraini Talip Mohd. Ruzi Abdul Rahman Nurul Hajar Razman 03.08.07
		NT 215	Hutan Simpan Endau Rompin, Johor	Noraini Talip Mohd. Ruzi Abdul Rahman 13.11.07
		NT216	Hutan Simpan Endau Rompin, Johor	Noraini Talip Mohd. Ruzi Abdul Rahman 13.11.07
	J. lanceolata	NT 97	FRIM Kepong, Selangor <i>Ex-situ</i> collection	Noraini Talip Mohd. Ruzi Abdul Rahman Nurul Hajar Razman 03.08.07
		NT230	Gunung Angsi, Negeri Sembilan	Noraini Talip Mohd. Ruzi Abdul Rahman 19.02.08
		RZ31	Gunung Angsi, Negeri Sembilan	Mohd. Ruzi Abdul Rahman 19.02.08
	J. magnifica	NT 99	FRIM Kepong, Selangor <i>Ex-situ</i> collection	Noraini Talip Mohd. Ruzi Abdul Rahman Nurul Hajar Razman 03.08.07
		NT232	Gunung Angsi, Negeri Sembilan	Noraini Talip Mohd. Ruzi Abdul Rahman 19.02.08
		NT244	Gunung Angsi, Negeri Sembilan	Noraini Talip Mohd. Ruzi Abdul Rahman 19.02.08
		RZ30	Empangan Semenyih, Selangor	Mohd. Ruzi Abdul Rahman 10.11.08
	J. perakensis	NT239	Kledang Saiong, Perak	Noraini Talip Mohd. Ruzi Abdul Rahman 21.02.08
		NT243	Kledang Saiong, Perak	Noraini Talip Mohd. Ruzi Abdul Rahman 21.02.08
		RZ33	Hutan Simpan Gunong Bubu, Perak	Mohd. Ruzi Abdul Rahman 10.10.08

APPENDIX 1 Details of the specimens of *Johannesteijsmannia* species examined