

MACROMORPHOLOGICAL AND MICROMORPHOLOGICAL STUDY OF SPATHES OF *Musa sapientum* L. AND *Musa paradisiaca* L. IN THE UNIVERSITY OF IBADAN

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Abstract. A comparative study of epidermal peels and transverse sections of spathes and rachis of *Musa sapientum* L. and *Musa paradisiaca* L. was carried out in search of constant microcharacters. The abaxial surfaces of the spathes of *M. sapientum* and *M. paradisiaca* showed similarities in parenchyma cell wall shape, presence of raphides, papillae base and stomata type, while there were marked differences in anticlinal walls and network of stomata on the abaxial surface. The adaxial surfaces of the spathes of the two species showed similar pattern in parenchyma cell wall and stomata type but differ in the anticlinal walls, raphide bundles and base of papillae. In addition, there were marked similarities in the trichomes on the rachis epidermis of *M. sapientum* and *M. paradisiaca*, with minor quantitative differences in distribution. *M. sapientum* and *M. paradisiaca* can be distinguished on the basis of the microcharacters on the spathes and rachis.

Keywords: Musa, epidermal, rachis, spathe, microcharacters

INTRODUCTION

The family *Musaceae*, made up of flowering plants, is placed in the order Zingiberales and assigned to the clade commelinids in the monocots [28]. The family is native to the tropics of Africa and Asia [30]. The centre of origin of the genus "*Musa*" is in South-East Asia [24]. Southeast Asia is the region of primary diversity of banana [10] while Areas of secondary diversity are found in Africa, indicating a long history of banana cultivation in the region [20].

The largest and most economically important genus in the family is "*Musa*", famous for the banana and plantain. The genus "*Musa*" was formally established in the first edition of Linnaeus' *Species Plantarum* [13]. This is the publication that marks the start of the present formal botanical nomenclature. When it was later realised that *Musa paradisiaca* Linn. – like *Musa sapientum* Linn. which Linnaeus had also added to the genus and was later recognized as a Silk banana – were hybrids between *M. acuminata* and *M. balbisiana*, some authors began to use the form *Musa* x *paradisiaca* and *Musa* x *sapientum* to emphasise that fact. The 1753 name [13], *M. paradisiaca* for plantains and *M. sapientum* for bananas are now known to refer to hybrids of these two species, rather than natural species. Today, it is well known that most cultivated seedless bananas are hybrids or polyploids of two wild banana species: *M. acuminata* and *M. balbisiana* [13]. Hybridization and polyploidy was the cause of much confusion in the taxonomy of the genus "*Musa*" that was not resolved until the 1940s and 1950s [21].

Banana and plantain has many worldwide uses including; Food [4, 3], Medicinal [16], Textile, Pulping, Arts [9, 22], Religion and Ornamental [2], Brewing [14] and Others including use for house roofs, umbrellas, plates, cattle feed, cigarette papers, clothing and packing materials; leaf buds for vegetables; leaf sheaths for water runways thatching, for stringing, tying, for plaiting into clothing and for cloth and thread. Ashes used for dyeing and tanning. Banana peel

used in extracting heavy metal contamination from river water in similarity to other purification materials [6].

The classification of cultivated bananas has long been a problematic issue for taxonomists. Linnaeus originally placed bananas into two species based on their uses as food: *M. sapientum* for dessert bananas and *M. paradisiaca* for plantains [13]. Subsequent further species names were added. However, this approach proved inadequate to address the sheer number of cultivars existing in the primary center of diversity of the genus, Southeast Asia. Many of these cultivars were given names which proved to be synonyms [30].

In a series of papers published in 1947 onwards, Ernest Cheesman [7] showed that Linnaeus's *M. sapientum* and *M. paradisiaca* were actually cultivars and descendants of two wild seed-producing species, *M. acuminata* and *M. balbisiana*, both first described by Luigi Aloysius Colla. He recommended the abolition of Linnaeus's species in favour of reclassifying bananas according to three morphologically distinct groups of cultivars- those primarily exhibiting the botanical characteristics of *M. balbisiana*, those primarily exhibiting the botanical characteristics of *M. acuminata*, and those with characters that are the combination of two [23].

Researchers Norman Simmonds and Ken Shepherd proposed a genome-based nomenclature system in 1955 in which plantain cultivars which are often cooked belong to the AAB group. This system of nomenclature eliminated all the difficulties and inconsistencies of the earlier classification of bananas based on assigning scientific names to cultivated varieties. Despite this, the original names are still recognized by some authorities today, leading to confusion [25].

The name *M. paradisiaca* for hybrids of *M. balbisiana* x *M. acuminata* was approved by the Royal Botanic Gardens [13]. The identification by Cheesman of *M. paradisiaca* with a specific "plantain" and *M.*

sapientum with a specific "banana" appeared to reinforce a prevailing tendency to associate the specific names with a specific type of fruit, respectively either a "cooking" plantain or a "dessert" banana was considered entirely semantic and artificial having no botanical basis at all nor indeed of any consistent culinary basis [13].

More so, over the years several authors have attempted to base the nomenclature of some *Musa* species on *M. paradisiaca* and *M. sapientum*. Sometimes, ignoring even botanical priority, *M. paradisiaca* was treated as a subspecies of *M. sapientum*. Sometimes, *M. sapientum* was treated as a subspecies of *M. paradisiaca*. These nomenclature systems were considered unsuccessful [13]. Various minor and major regroupings have been suggested [8, 31].

However, researchers have attempted to differentiate between these two species of the genus *Musa*, *M. sapientum* and *M. paradisiaca*, based on morphology, size, colour, taste, consumption, uses and skin texture, with little or no success, therefore the need to improve our knowledge on *M. sapientum* (banana) and *M. paradisiaca* (plantain) through the anatomical studies of the microstructures of their spathes using epidermal peels, transverse sections and trichomes. This study is aimed at examining old data and providing new information with a view to improving our knowledge of *M. sapientum* and *M. paradisiaca* using anatomical characters of the spathes and trichomes.

MATERIALS AND METHODS

Fresh spathes and rachis of *M. sapientum* (banana) and *M. paradisiaca* (plantain) numbering up to 15 samples each were collected from six different locations within Ibadan city, most especially in the University of Ibadan and fixed in 50% alcohol. The spathes were then prepared for epidermal peeling and transverse sectioning, while the scrapings off the rachis were prepared for microscopy. There was a complete histological preparation of the spathes using a modification of the [12] method of slide preparation; sectioning was done using a microtome, dehydrated in a series of ethyl alcohol 70%, 80%, 90% and 95% while staining was done using Safranin O as primary stains while Alcian blue and Lactophenol was used as secondary stain. Buechler's method of clearing the leaves [5a] was followed before the epidermal surfaces of the spathes were peeled and subsequently stained using Safranin O. The sections and epidermal peels, and scrapings off the rachis were then attached firmly on separate slides and analysed under various resolutions of the microscope. Photomicrographs were recorded using a high sensitivity colour camera system which allows generation of an in-focus composite image from up to ten images recorded by merging their sharply focused regions together.

RESULTS

The results of the photomicrographs of prepared slides of the spathes of *M. paradisiaca* and *M. sapientum* are as reported below;

Abaxial Epidermis Of Spathes

The shape of the parenchyma cell on the abaxial epidermal peels of spathes of both *M. sapientum* and *M. paradisiaca* is predominately polygonal (Fig. 1A). In some cases parenchyma cells are arranged in tiles (Figs. 1G). Raised base of papillae are present on the abaxial surface of the epidermal peels of *M. paradisiaca* and *M. sapientum* (Figs. 1B, 1E and 1F), and raised anticlinal walls found on the epidermis of the spathes of some of the *M. sapientum* (Fig. 1B). Raphides are present on the abaxial epidermal peels of the spathes of both species (Figs. 2E and 1I), but fewer on those of *M. paradisiaca*. The raphides bundles found on these surfaces are elongated, needle-like, crystal clear structures with tapering sharp edges (Fig. 1F). Raphides do not absorb colour due to their calcium oxalate content. Each raphides bundle is enclosed in a membrane-like envelope called raphidiosomes (Fig. 1I). However, some of these membrane structures were seen to have burst and their contents (the raphides) liberated onto the surface (Fig. 1F). Paracytic stomata surrounded by raised stomata sheath are present on the abaxial epidermal peels of both *M. sapientum* and *M. paradisiaca* (Figs. 1C, 1D, 1H). There was also a case of networked stomata (Fig. 1C). The subsidiary cells surrounding the guard cells of the networked stomata in the spathe of *M. sapientum* vary in number (between 2 and 3) and are sub-equal. Tracheary element is also seen on the abaxial epidermis of *M. sapientum* (Fig. 1E).

Adaxial Epidermis Of Spathes

The parenchyma cells on the adaxial epidermal peels of spathes of both species are predominantly polygonal in shape; varying from 4 sides to 7 sides (Figs. 2A and 2E). There are also instances whereby the parenchyma cell walls arranged in tiles (Fig. 2E). Raised anticlinal walls are present on the adaxial surfaces of the spathe of *M. sapientum* (Fig. 2B), but absent on those of *M. paradisiaca* (Fig. 2E). Also, base of papillae are present on the adaxial surface of *M. sapientum* (Fig. 2B), whereas, it is absent on that of *M. paradisiaca* (Fig. 2E). Raphides are present on the adaxial epidermal peels of the spathes of *M. sapientum* (Fig. 2C), but absent on those of *M. paradisiaca* (Fig. 2E). The raphides are enclosed in a membrane-like envelope known as raphidiosome. Abundant paracytic stomata are present on the adaxial surface of the epidermal peels of spathes of both *M. sapientum* and *M. paradisiaca* (Figs. 2D and 2E). Each stoma is surrounded by raised stomata sledge (Fig. 2F).

Trichomes

The parenchyma cells that made up the surface of the rachis are rectangular in shape (Fig. 3C). Simple unicellular trichomes are found on the epidermal layer of the rachis of both *M. sapientum* and *M. paradisiaca*

(Fig. 3B and 3D). However, their relative abundance and length varies (Figs. 3A and 3C). The trichomes found on the rachis of *M. paradisiaca* are relatively shorter and fewer (Fig. 3D) compared with those found on the rachis of *M. sapientum* (fig. 3A).

Transverse Sections

The shape of the parenchyma cells across the transverse sections of both *M. sapientum* and *M. paradisiaca* are varied (Fig 4B). There are hollow centers/empty spaces (lacunae) in each of the transverse sections of both *M. sapientum* and *M.*

paradisiaca. However, the size, shape and structure of the lacunae differ. (Figs. 4A, 4G, 4H, 4I and 4J). Tracheoidal idioblasts, protoxylem and metaxylem are also present in both species (Figs. 4C), but the differentiation and organization of the vascular bundles is poor. Also, there is no noticeable phloem (Figs. 4F and 4I). Xyllary fibres, and fibres are also present in the transverse sections of both *M. sapientum* and *M. paradisiaca* (Figs. 4C and 4F). Pappilae (temporary growths found on the outermost layer (i.e. epidermis) of a cell) are found on the epidermis of the transverse

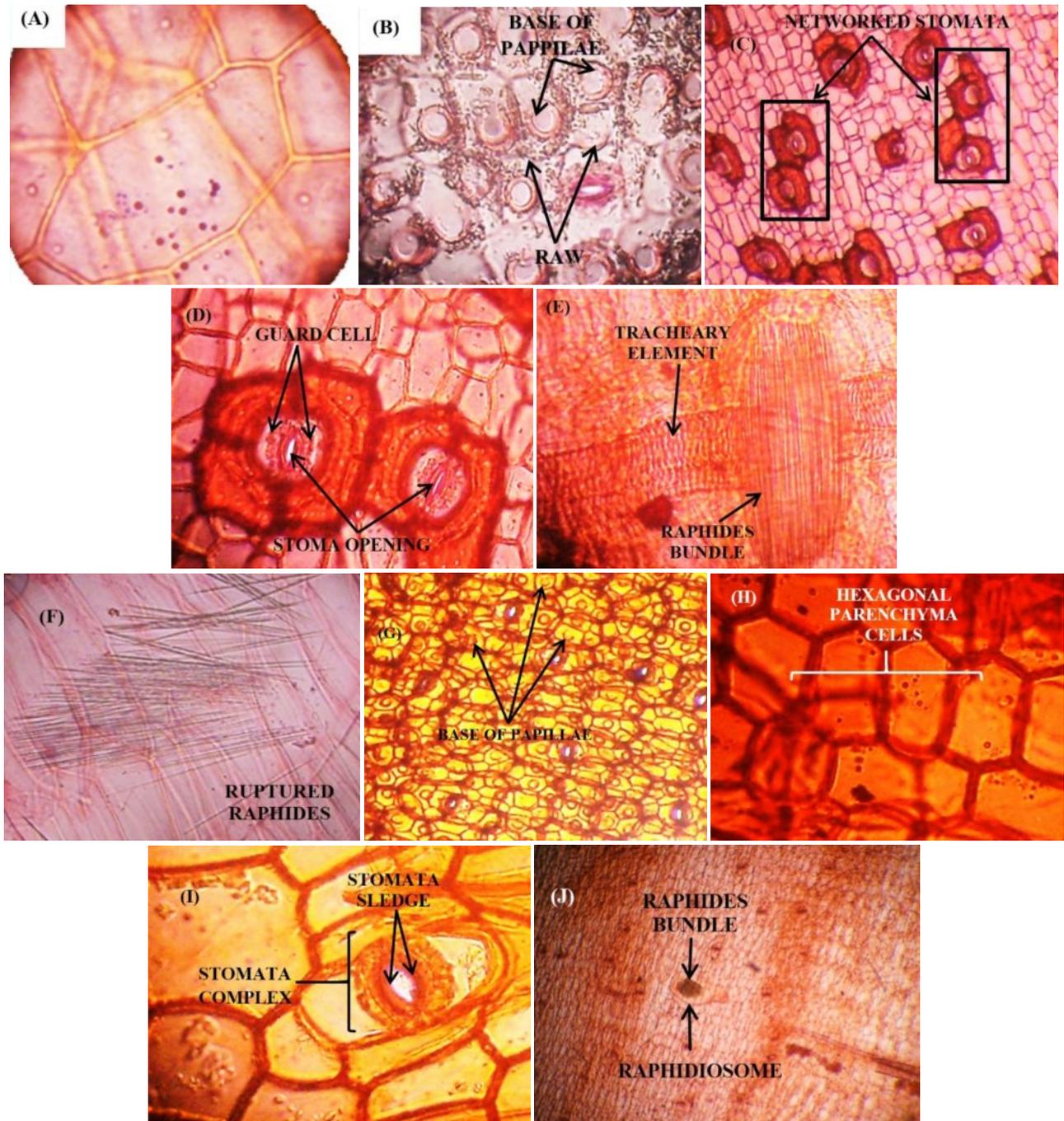


Figure 1. (A-F) Abaxial epidermis of the spathes of *M. sapientum*; (A) Polygonal cell (B) Raised anticlinal walls and base of papillae (C) Networked stomata (D) The stomata opening, guard cells and complimentary cells of a stoma (E) Raphides bundle enclosed in a raphidiosome, and tracheary element (F) Bursted raphides with sharp edges; Figs. 1(G-K) Abaxial epidermis of the spathes of *M. paradisiaca* (G) The base of papillae (H) Tile arrangement of hexagonal parenchyma cell (I) Stomata complex made up of complimentary cells, stomata sledge, guard cells (J) Raphides bundle enclosed in a raphidiosome

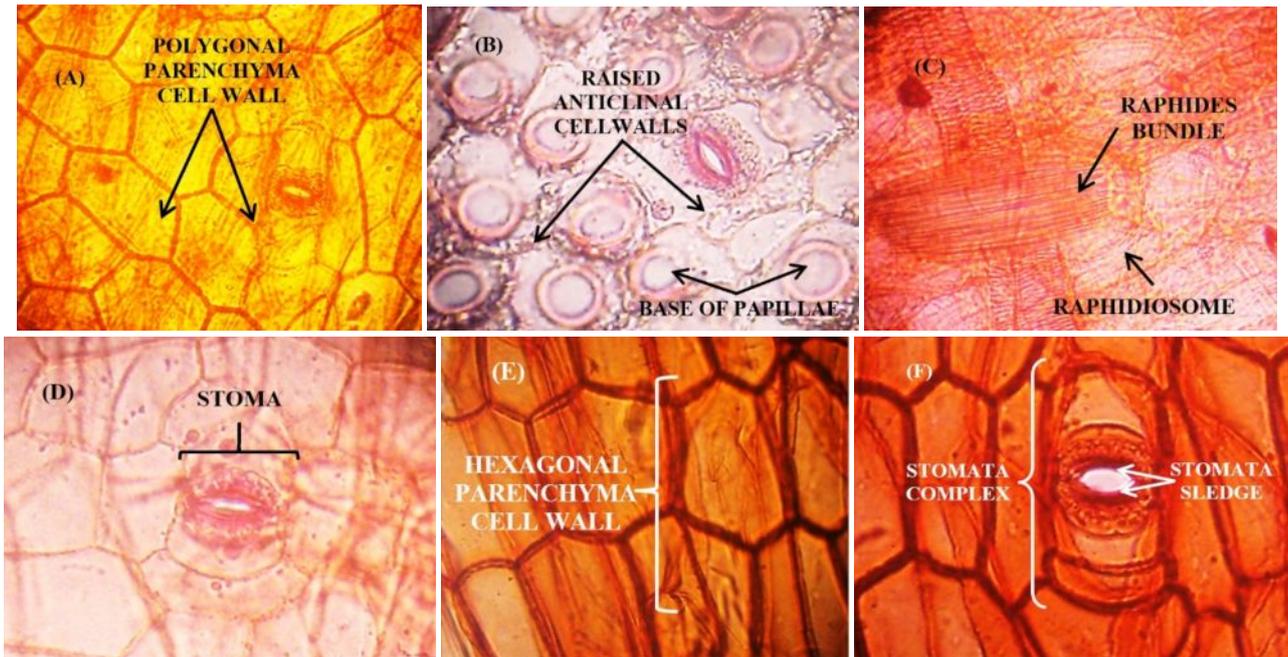


Figure 2. (A-D) Adaxial epidermis of the spathes of *M. sapientum*; (A) Polygonal-shaped parenchyma cell walls (B) Raised anticlinal walls and base of papillae (C) Raphides bundles enclosed in raphidiosome (D) stomata complex; (E-F) Adaxial epidermis of the spathes of *M. paradisiaca* (E) Tile arrangement of hexagonal-shaped parenchyma cell walls (F) Stoma complex showing the stoma sledge.

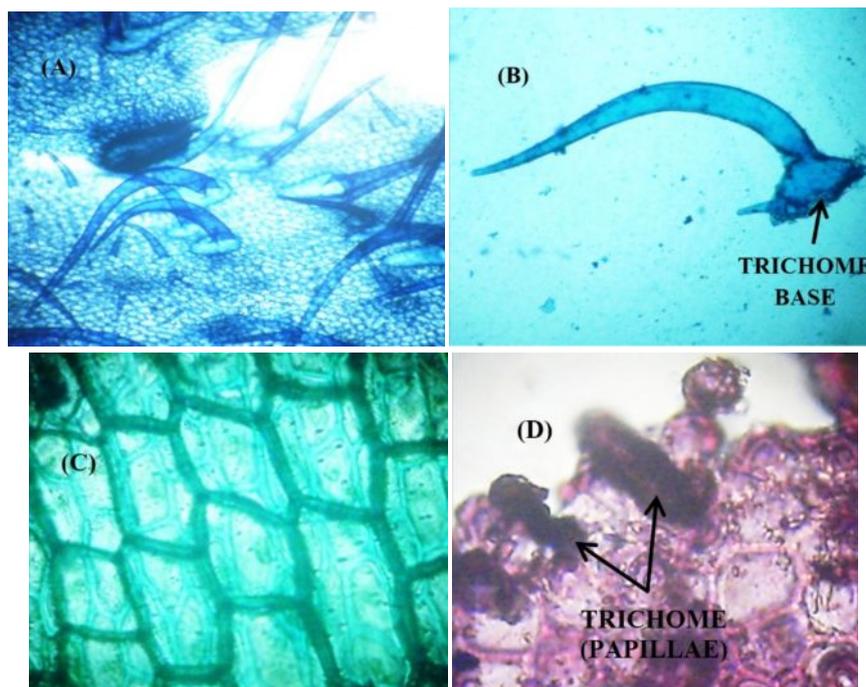
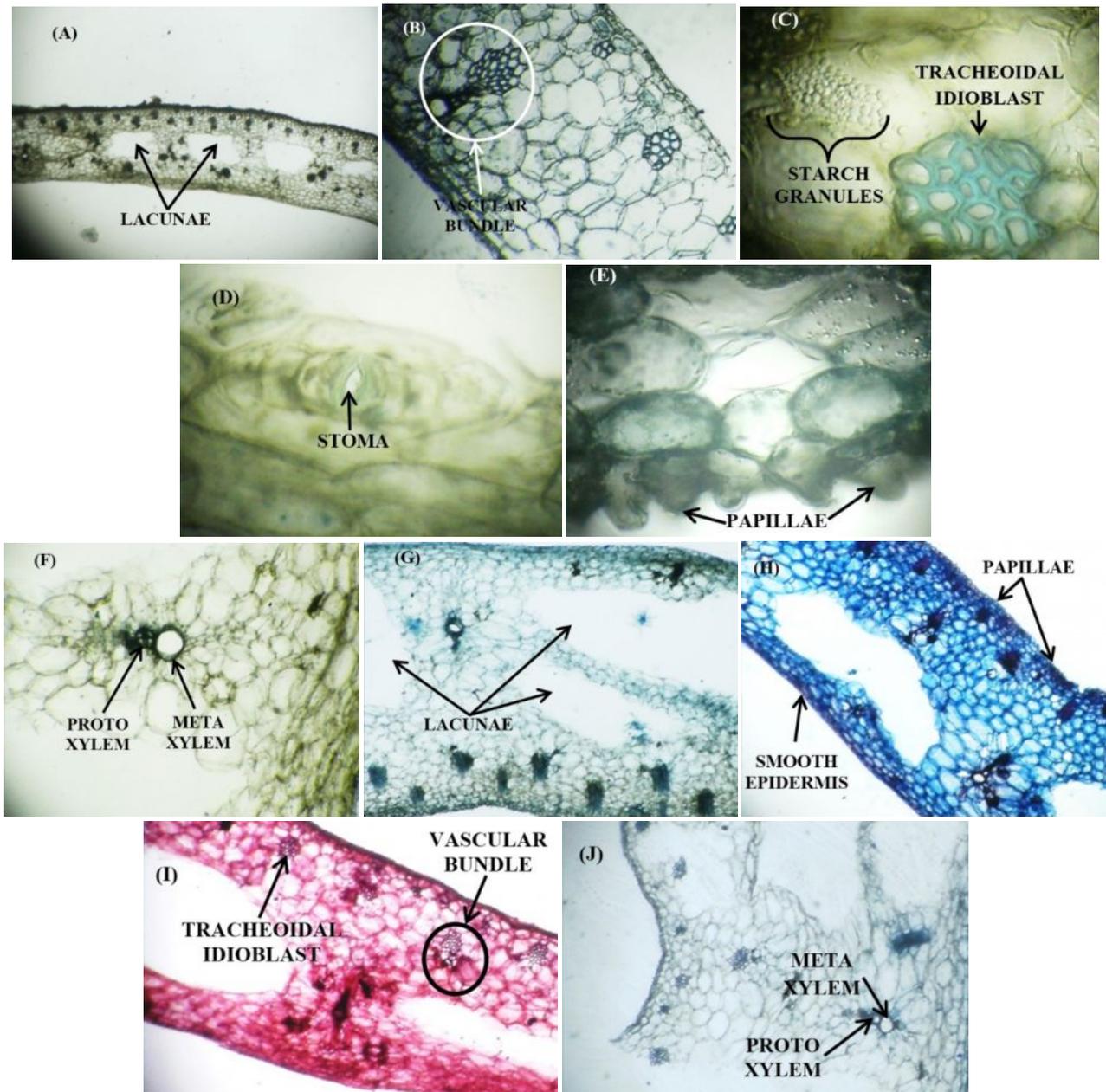


Figure 3. (A-D) Peel of the rachis of *M. Sapiantum* and *M. paradisiaca*; (A) Overview of trichomes on the surface of the rachis of *M. sapientum* (B) A typical simple unicellular trichome found on the surface of the rachis of *M. sapientum* (C) Overview of the surface of rachis of *M. paradisiaca* (D) A typical simple unicellular trichome found on the surface of the rachis of *M. paradisiaca*

sections of spathes of both *M. sapientum* and *M. paradisiaca* (Figs.4E and 4H). However, papillae on the spathes of *M. sapientum* were denser than those found on the spathes of *M. paradisiaca*. Moreover, these papillae occur on both surfaces of the epidermis of the transverse sections of *M. sapientum* (Figs. 4A and 4B), whereas it was found on only one surface (abaxial) of those of *M. paradisiaca* (Fig. 4F). Other differences

observed include presence/absence of stoma and starch granules. Stomata are present in the transverse sections of the spathes of *M. sapientum* (Fig. 4D) and absent in those of *M. paradisiaca* (Figs. 4 E-H). Also, starch granules are present in the transverse sections of spathes of *M. sapientum* (Fig. 4C) and absent in those of *M. paradisiaca* (Figs. 4E-H).



Figures 4. (A-F); Transverse sections of the spathes of *M. sapientum* showing (A) Lacunae (B) Vascular bundle and varied parenchyma cell wall (C) Starch granules and tracheoidal idioblast (D) Stomata (E) Papillae (F) Protoxylem and metaxylem; (G-J): Transverse sections of the spathes of *M. paradisiaca* showing (G) Lacunae (H) Smooth epidermis and papillae on the same transverse section (I) Vascular bundle and tracheoidal idioblasts (J) Protoxylem and metaxylem

DISCUSSION

After careful and thorough examination of the micrograph pictures of prepared slides taken, the following inferences were drawn.

The similarities between the abaxial surfaces of the epidermal peels of *M. sapientum* and *M. paradisiaca* include; presence of open paracytic stomata with raised stomata sledge, raised polygonal parenchyma cell wall, base of papillae in line with Tomlison and Osuji [19, 29]. The work of Angeles *et al.* [1] however reported the presence of tetracytic stomata; Another similarity is the presence of raphides bundles which stores calcium oxalate; a poisonous chemical compound which may be a form of defense against other preying

organisms [15]. The calcium oxalate crystals are characteristically similar to those found in Curcubitaceae [27] and Dioscoraceae [28]. However, the differences that occur include; the presence of more raphides bundles on the abaxial surface of *M. sapientum* than that of *M. paradisiaca*, presence of anticlinal walls on the abaxial surfaces of *M. sapientum* which were absent on the abaxial surfaces of *M. paradisiaca* and networked stomata which were not present on any of the epidermis of the spathes of *M. paradisiaca* sample collected were present on the abaxial surface of *M. sapientum*. The networks involved two to three to four and five stomata with joint subsidiary cells. This was not accounted for in previous literatures, with [27] reporting basically the

size and distribution of the stomata. The presence of raised anticlinal wall is a unique character because not even all the *M. sapientum* samples collected have it, this may be due to the environment, as environment plays a huge in make some anatomical features plastic. The presence of raphides on the abaxial surface of *M. paradisiaca* is however in contrast with the report of Osuji [19] which says that, “raphides are absent on the epidermis of *M. paradisiaca*”.

Similarities noted in the micrograph of the adaxial surfaces of spathes of *M. sapientum* and *M. paradisiaca* includes; polygonal shaped parenchyma cell wall and presence of abundant paracytic stomata surrounded by stomata sledge. The raised stomata sledge was not reported in the work of Osuji, and Sumardi and Wulandari [19, 27]. However, differences between the adaxial surface of spathes of *M. sapientum* and those of *M. paradisiaca*. include; presence of anticlinal walls on the adaxial surface of *M. sapientum* which were absent on adaxial surfaces of spathes of *M. paradisiaca*, the presence of raphides on the adaxial epidermal peels of spathes of the spathes of *M. sapientum* and its absence on those of *M. paradisiaca* and also, the presence of base of papillae on the adaxial surface of spathes of *M. sapientum* and its absence on those of *M. paradisiaca*.

The presence of papillae at both edges (i.e. the abaxial and adaxial surfaces) of the transverse sections of the spathes of *M. sapientum* is in line with the report of Osuji, [19]. However, papillae were only present at one edge (adaxial surface) of the transverse section of *M. paradisiaca* while the other edge (abaxial surface) was smooth. This is in line with the work done by Osuji [19] in which he stated that; “the transverse sections “the abaxial epidermal surface of *M. sapientum* showed the presence of papillae in virtually every cells, but not obvious on the abaxial surface of *M. paradisiaca*”. However, in contrast, the density of papillae was not recorded. In this work, it was noted that the papillae on *M. sapientum* are denser than the papillae found on *M. paradisiaca*.

The vascular bundles found in the transverse sections of the spathes of *M. sapientum* and *M. paradisiaca* are poorly differentiated and so not well organized. More so, the vascular bundles were scattered, thereby confirming that the two species i.e. *M. sapientum* and *M. paradisiaca* are monocotyledonous plants [30, 31]. This may be as a result of less need for them to conserve, but to conduct water and other mineral elements since the major function the spathes serve is to serve as a protection in form of covering to the young flowers. The vascular bundles found in the two species are made up of; xylary bundles, protoxylem, metaxylem and tracheoidal idioblasts [30]. However, there was no noticeable phloem present in the transverse sections. There was no record of starch granules in the report of the work of Osuji, [19]. However, starch granules were seen on the transverse section of the spathe *M. sapientum* in this work. This shows that, *M. sapientum*

stores its excess food in form of starch; a product of photosynthesis.

The type, presence, absence and location of trichomes are important diagnostic characters in plant identification and taxonomy [26]. In difference from Osuji [19], trichomes from the rachis of *M. sapientum* and *M. paradisiaca* were observed in this work. It was discovered that trichomes present on the rachis of *M. sapientum* and *M. paradisiaca* are simple and unicellular. However, the trichomes found on the rachis of *M. paradisiaca* were relatively shorter and fewer [19].

The spathes of *M. sapientum* and *M. paradisiaca* have very similar macromorphological features and characteristics irrespective of the various habitats from which they were collected. In spite of this, environmental factors are not yet known to affect spathe morphology in *M. sapientum* and *M. paradisiaca*. The use of anatomical characters such as arrangement and number of sides of their parenchyma cells, raised anticlinal walls, stomata, papillae, xylary fibres, protoxylem, metaxylem, tracheal idioblasts, raphides idioblasts and lacuna are not very useful in distinguishing *M. sapientum* and *M. paradisiaca*. This is due to the fact that, in respect of this particular study and previous studies, anatomical characters of the spathes of *M. sapientum* and *M. paradisiaca* have shown very little variations. This calls for more work in this particular field, perhaps employing the use of molecular data and samples from other locations for the purpose of accuracy and to avoid missing data.

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