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## Microstructural Characters of the Inflorescence Bracts Discriminate Between *Musa sapientum* L. and *M. paradisiaca* L.

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**Abstract:** Epidermal and anatomical features of the inflorescence bracts of *Musa sapientum* L. and *M. paradisiaca* L. were studied using epidermal peeling and wax-embedding techniques. The organization of the ground tissue, stele and number of tiers of the epidermal cells of the bracts did not distinguish *M. sapientum* from *M. paradisiaca*. Paratrachytic, brachyparatrachytic and brachyparaxyletic-monopolar stomata were present in the bracts of both species. In contrast, the presence of large raphide idioblasts, each containing a raphide bundle, on the adaxial epidermis as well as occurrence of papillae on the abaxial epidermis of *Musa sapientum* and their absence from the bracts of *M. paradisiaca* were very discriminatory between the two closely related species. The raphide bundle in the idioblast measured 54-76 µm long and 48-72 µm thick. Evidence from these features of the bract showed that these epidermal characters were diagnostic and may be employed in the taxonomic treatment of these species as taxonomically distinct taxa as well as in the characterization of the *Eumusa* section of the genus *Musa* L.

**Key words:** Epidermis, inflorescence bracts, idioblasts, raphide bundles, papillae, *Musa* spp.

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### INTRODUCTION

The genus *Musa* L. (family, Musaceae; order, Zingiberales) originated in Asia but has become widely distributed throughout the tropics (Champion, 1963; Cobby and Steele, 1977). In West Africa, the cultivated representatives of this genus were derived from two Asiatic diploid species, *M. acuminata* Colla AA and *M. balbisiana* Colla BB. The hybrid cultivars are usually represented as AA, BB, AAA, AAB, etc. depending on the dosage of genomic material inherited from the parental diploids.

Parthenocarpic *Musa*, which comprises bananas and plantains were transferred by man to South America and East Africa from where they spread to Central and West Africa. East Africa became the secondary centre of diversification of AA and AAA bananas (*sensu stricto*) while West and Central Africa were the secondary centres of diversification of plantains AAB (Simmonds, 1966).

The diversification of the edible *Musa* was reportedly due to somatic mutations (Samson, 1982) and somaclonal variations (Vuylsteke *et al.*, 1991). Owing to

the prevalence of genetic instability, the consequent wide phenotypic diversity, their hybrid origin and the wrong determination of nomenclatural type for *M. sapientum* (Samson, 1982), the specific status of the two *Musa* species were being doubted. Hence Shepherd (1990) avoided the mention of these two species in his observations on *Musa* taxonomy. Similarly, Swennen (1990), in his account of the limits of morphotaxonomy of plantains in Africa and elsewhere avoided the use of the nomenclature *Musa paradisiaca* for plantain and used the genotypic description AAB to represent the taxon. In addition, the wide array of phenotypic variability in the germplasm made difficult, the separation of the triploid cultivars into clearly different species (Simmonds and Shepherd, 1955). Consequently, reports on taxonomic research on *Musa* (Swennen *et al.*, 1995; Simmonds and Weatherup, 1988, 1990; Swennen and Vuylsteke, 1987) avoided the use of the names *M. paradisiaca* for plantain and *M. sapientum* for banana. Rather, *Musa* spp., AAA group, *Musa* spp., AAB group, etc. are frequently being used.

Stace (1980) reported the conservativeness of anatomical characters in taxonomy since such characters

are virtually unaffected by environmental changes. Obviously, micromorphological and anatomical characters have not been vividly exploited in the taxonomic treatment of this genus. Micromorphological and anatomical features have been used with high diagnostic values in the taxonomic evaluation of some plant species. Anatomical structures including epidermal features have been reported to yield very important taxonomic information (Carlquist, 1961). Taxonomic problems have been solved using microstructural characters in the several taxa (Foreman, 1966; Martin, 1954; Nyananyo, 1989; Okoli, 1989; Stace, 1980). Though the use of such features in settling taxonomic disputes has not been conducted to my knowledge, such factors could produce very conservation evidence(s) useful in their taxonomic treatment.

Due to a need to produce diagnostic evidence that the two species are taxonomically distinct, this work was conducted to show that micromorphology and anatomy of the inflorescence bracts could be useful in delimiting banana, *Musa sapientum* L., widely referred to by breeding programmes as *Musa* spp. AAA group from plantain, *Musa paradisiaca* L., otherwise referred to by breeding programmes as *Musa* spp., AAB group.

#### MATERIALS AND METHODS

The materials used for this work were obtained from the IITA *Musa* germplasm located at Onne, near Port Harcourt and taken to the University of Port Harcourt, Nigeria where this work was conducted. The cultivars studied include three plantains (Agbagba, Mbi Egome and Bobby Tannap), three East African Cooking bananas (Rugondo, Nakitengwa and Igitsiri) and two Dessert bananas (Valery and Robusta; Table 1). Epidermal strips were obtained from the adaxial and abaxial surfaces of the bracts and stained lightly with 0.5 % safranin T solutions.

Anatomical studies were carried out on bracts, which were collected in Formal acetic acid ethanol (1:1:18 v/v) solution for 48 h, dehydrated with ethanol series, infiltrated with ethanol/xylene series according to the method of Osuji *et al.* (1995). Micrometric sections were stained with 0.5% alcian blue (for 5 min) and counter-stained with 0.5% safranin T solution (for 3 min). All micrographs were taken from good preparations using Leitz Diaplan photo-microscope fitted with Leica WILD MPS 52 camera.

Table 1: Plantain and banana cultivars studied

Cultivar	Genotype	Group	Sub-group
Agbagba	AAB	Plantain ( <i>Mp.</i> )	Medium French
Mbi Egome	AAB	Plantain ( <i>Mp.</i> )	Small French
Bobby Tannap	AAB	Plantain ( <i>Mp.</i> )	Medium French
Rugondo	AA	Banana ( <i>Ms.</i> )	East African Cooking
Nakitengwa	AAA	Banana ( <i>Ms.</i> )	East African Cooking
Igitsiri	AAA	Banana ( <i>Ms.</i> )	East African Cooking
Robusta	AAA	Banana ( <i>Ms.</i> )	Dessert
Valery	AAA	Banana ( <i>Ms.</i> )	Dessert (Cavendish)

*Mp.* = *Musa paradisiaca* L.; *Ms.* = *Musa sapientum* L.

#### RESULTS

The bracts of all cultivars were dorsiventral with smooth adaxial and corrugated abaxial surfaces. The adaxial epidermis of the bracts of *M. paradisiaca* cultivars was glabrous and had numerous paratracytic, brachyparatracytic and brachyparahexacytic-monopolar stomata (Fig. 1). On the other hand, the adaxial epidermis of the bracts of *M. sapientum* cultivars had raphide idioblasts each of which contained a raphide bundle in addition to the numerous paratracytic, brachyparatracytic and brachyparahexacytic-monopolar stomata. Frequently, a jellylike substance surrounded the raphide bundle in the idioblast. Each bundle in the idioblast measured 54-76  $\mu$ m long and 48-72  $\mu$ m thick. Both raphide idioblast and the inclusive bundle were absent from the adaxial epidermis of the bracts of *M. paradisiaca*.

The abaxial epidermal surface of *M. sapientum* cultivars showed the presence of papillae in apparently every cell (Fig. 1). The papillae were not obvious in the abaxial epidermis of *M. paradisiaca*. But there were obvious signs of encrustations of cytoplasmic material on the abaxial epidermal cells of *M. paradisiaca*. The abaxial epidermis of all the cultivars contained unidirectionally arranged stomata, which were similar to those on the adaxial epidermis.

Transverse sections (Fig. 2) revealed clearly, the numerous papillae on the abaxial surface of the bracts of *M. sapientum*. There were no such structures on the abaxial surface of the bract of *M. paradisiaca* cultivars. These papillae measured 12-20  $\mu$ m both in length and width. The base of each papillae opened into the adjoining epidermal cell.

The ground tissue of the bract in both species is partitioned into two dorsiventral regions by large laterally arranged lacunae (Fig. 2). The ground parenchyma in *M. sapientum* bracts was more angular than that of *M. paradisiaca*. The vascular bundles were dorsiventrally elongated but occasionally appeared radial. One large or

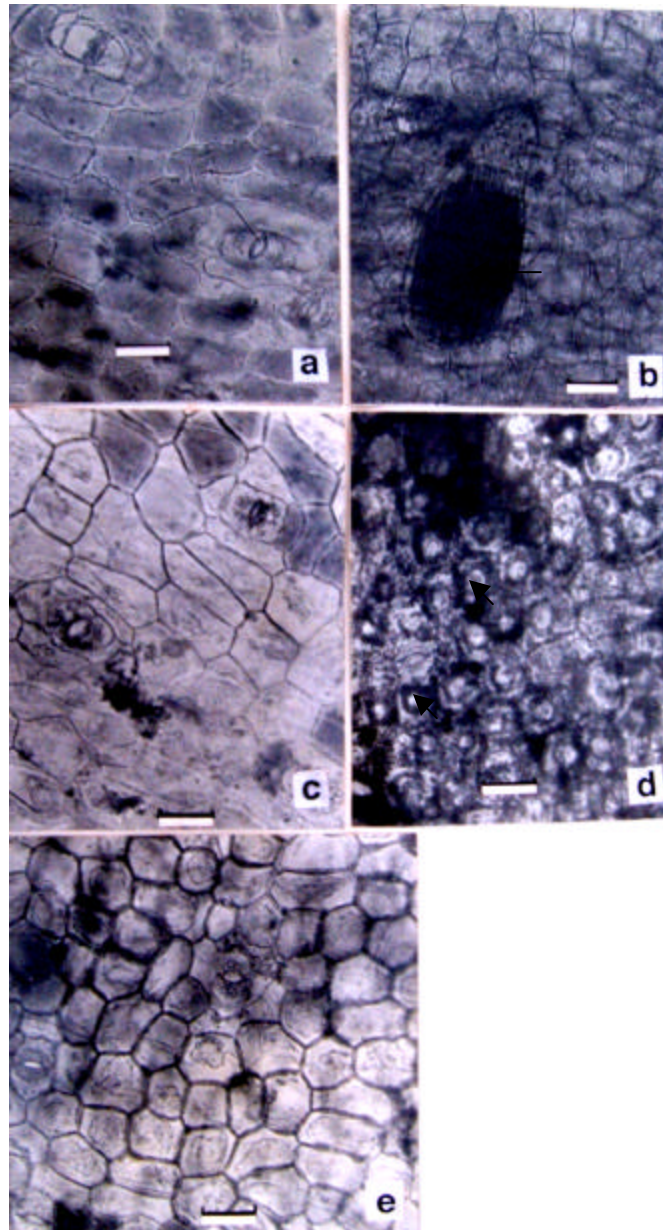


Fig. 1: Epidermal characteristics of the bracts of *M. sapientum* and *M. paradisiaca*. a-b) Adaxial epidermis of *M. sapientum* cultivars; a) 'Valery' showing the two types of stomata; b) 'Nakitengwa' showing a raphide bundle localized in a raphide idioblast; c) Adaxial epidermis of *M. paradisiaca* cultivar 'Mbi Egome'; d) Abaxial epidermis of 'Nakitengwa' showing the outlines of the bases of papillae; e) Abaxial epidermis of 'Mbi Egome' indicating cytoplasmic encrustations on the epidermal cells. Note that trichomes and/or glands were absent. Scale bar = 40  $\mu$ m

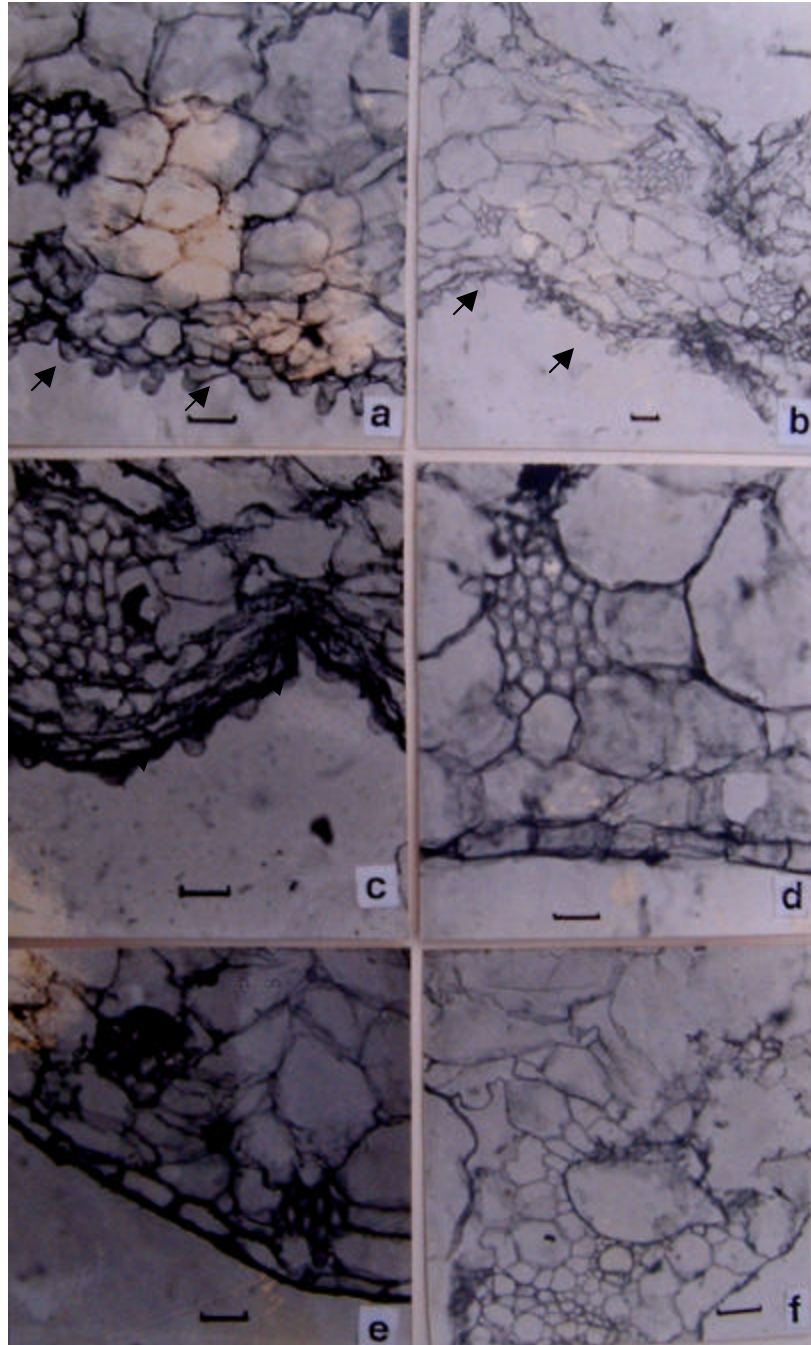


Fig. 2: Transverse sections showing the presence of papillae on the abaxial surface of the bracts. a-c) *M. sapientum*; a) 'Rugondo, b) Robusta, c) Igitsiri; d-e) *M. paradisiaca*; d) Agbagba, e) 'Bobby Tannap, f) a vascular bundle. Scale bar = 40  $\mu$ m

two moderately sized lysigenous cavities marked the position of the xylem vessels (Fig. 2) while the phloem position was made distinct by a collection of phloem fibres.

## DISCUSSION

The taxonomic separation of *M. sapientum* L. from *M. paradisiaca* L. has been a major cause for concern in *Musa* breeding programmes. Consequently, *Musa* taxonomists had resorted to the use of genotypic descriptions to delimit the constituent taxa in the *Musa* germplasm (Simmonds and Weatherup, 1988; Shepherd, 1990; Swennen, 1990; Swennen *et al.*, 1995; Vuylsteke *et al.*, 1991). This situation arose due to unavailability of more conservative data for germplasm characterization. The discriminative importance of anatomical features in taxonomic delimitations (Stace, 1980) can not be neglected because such features, when investigated on taxa maintained in the same environment under the same growing conditions, exhumed the true pattern of relationship between the affected taxa (Swennen, 1990). In addition to the employment of morphological attributes in the characterization of the *Musa* genus, molecular cytogenetic characterization had been employed to distinguish between the two widely cultivated triploid *Musa* species being investigated (Osuji *et al.*, 1997). Osuji *et al.* (2000) reported the histochemical variability in the structure of intra-amylar crystals of calcium oxalate between *M. sapientum* and *M. paradisiaca* but no anatomical evidence has been presented, in corroboration, to consolidate the vegetative dissimilarity between these cultivated species (or genotypes).

Evidences from this work agree with earlier observations and reports on the very important role features of the epidermis play in taxonomic delimitations (Stace, 1980; Nyananyo, 1989; Okoli, 1989). Great similarity was reported to exist between foliar stomatal structure in all groups and subgroups of cultivars and wild species in the *Musa* genus (Osuji, 1995). This similarity in the types of stomata in *Musa* subgroups indicates very close phylogenetic relationship between the constituent taxa in this genus. However, the pattern of occurrence and distribution of the different types of stomata discriminate more distinctly between the two wild diploid species *M. acuminata* and *M. balbisiana*, than their derived triploid cultivated species (Osuji, 1995).

Though stomatal features are to some extent discriminatory, they do not have clearly diagnostic value in the *Musa* genus. On the other hand, the occurrence of

papillae on the abaxial bract surface of *M. sapientum* and their absence in the bracts of *M. paradisiaca* is obviously diagnostic and could be solely used to distinguish between the two triploid *M. sapientum* (AAA) and *M. paradisiaca* (AAB). This situation therefore agrees with the opinion of Carlquist (1961) and Stace (1980) that epidermal features could be taxonomically diagnostic. This evidence of microstructural discontinuity also supports the treatment of *M. sapientum* and *M. paradisiaca* as distinct species.

The taxonomic value of occurrence and distribution of calcium oxalate crystals in the Cucurbitaceae (Okoli, 1988) and Dioscoreaceae (Okoli and Green, 1988) families is applicable to these two triploid *Musa* species in the Musaceae family due to the differential occurrence of raphide idioblasts. The occurrence of these microstructures in the adaxial epidermis of *M. sapientum* and their absence in *M. paradisiaca* corroborates other data in support of the taxonomic discontinuity between the two species of *Musa* L. This evidence agrees with the suggestion by Carlquist (1961) that anatomical structures including epidermal features yield very important taxonomic information.

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