

A Study on the Epicuticular Wax Morphology of African Acacias

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Abstract. Epicuticular waxes on the leaflets of 26 African acacias were examined using Scanning Electron Microscope (SEM) in an attempt to outline more characters that might be taxonomically significant. Three different epicuticular wax deposits (amorphous to weakly undulated wax, rosettes form of wax platelets and ridge-like wax platelets) were encountered to exist and five leaflet-types were recognized in the genus *Acacia*. These leaflet-types differ mainly in the epicuticular wax deposits, shape of epidermal cells and the distribution of trichomes on the surface of the leaflets. These types coincide with the previous primary divisions of the genus and suggested further subdivisions to accommodate the inter-specific wax variation. The study suggested that epicuticular wax deposits on the adaxial surfaces may, in conjunction with other characters, serve as good taxonomic tool for delimiting taxa.

Keywords: *Acacia*, Epicuticular wax, Scanning Electron Microscope (SEM), Epidermis morphology, Taxonomy.

Introduction

Acacia Mill. is a cosmopolitan genus of more than 1350 species and together with the monotypic genus *Faidherbia* (Del.) A. Chev., comprises Tribe Acacieae within Subfamily Mimosoideae [1]. In Africa, the genus *Acacia* is represented by 115 species [2].

Since it was first described by Philip Miller in 1754, the genus *Acacia* was subjected to a number of taxonomic treatments (mainly splitters) and continuous redefinition by various authors.

Bentham [3] divided the genus *Acacia* into six series, namely: Gummiferae, Vulgares, Filicinae, Phyllodineae, Botrycephalae and Pulchellae. African *Acacia* species fell into only two series: series Gummiferae (with spinescent stipules) and series Vulgares (with non-spinescent stipules).

Vassal [4] divided the genus into three subgenera, namely: *Acacia*, *Aculeiferum* and *Heterophyllum* (= *Phyllodineae*). The subgenera *Acacia* (= series *Gummiferae*) and *Aculeiferum* (= series *Vulgares*) comprise African *Acacia* species.

Pedley [5] proposed that Vassal's [4] three subgenera, should be treated as distinct genera, namely: *Acacia*, *Senegalia* and *Racosperma*. The African Acacias belong to the genera *Acacia* (= subgenus *Acacia* = series *Gummiferae*) and *Senegalia* (subgenus *Aculeiferum* = series *Vulgares*).

Pedley's [5] treatment (generic classification) was not widely adopted by subsequent botanists, but Maslin [6] and Vassal [7] agreed that the genus *Acacia* is polyphyletic and is in need of further classification.

Epicuticular wax covers the outer surfaces of most terrestrial plants and appears as crystallized bodies or amorphous layers in a great diversity of form and thickness [8]. Epicuticular wax ultra structures are not only essential elements of the surface, but also of fundamental functional and ecological importance for the interaction between plants and their environments [9]. Epicuticular wax ornamentation is of great systematic significance in understanding the relationships among species [10]. Moreover, Hallam and Chambers [11] demonstrated the potential of epicuticular studies to aid the classification of a complex genus.

Previous work on the epicuticular wax of various members of the genus *Acacia* is scarce. Metcalf and Chalk [12] described the outer walls of the epidermal cells of the genus *Acacia* as with ridge-like external projections. Bleckmann *et al.* [13] described the epicuticular wax crystals of *Acacia greggii* as forming discrete tufts. Baker [14] reported the presence of plates on *Acacia* leaves. No attempts were encountered so far to survey the epicuticular wax of the African members of the genus and its usefulness or otherwise in species clustering.

The main objective of the present work is to use Scanning Electron Microscope (SEM) to characterize the epicuticular wax morphology of selected African members of the genus *Acacia*, in search of taxonomic micro-characters that might assist in the identification and understanding of the inter-specific variations in the genus.

Materials and Methods

The leaflets of a total number of 26 *Acacia* specimens were collected from herbarium materials. A list of these specimens is presented in Table 1.

Small pieces (about 7 mm²) of the mature leaflets from the central adaxial surfaces of each species under examination were transferred directly to aluminum stubs with double adhesive tape. No attempt was made to remove foreign matters from the surfaces of the leaflets so as not to damage the epicuticular wax surface.

Table 1. Specimens investigated

1	<i>Acacia albida</i> Del. (= <i>Faidherbia albida</i> (Del.) A. Chev.). SUDAN: Zalingei. Wickens 1496 (KHU).
2	<i>A. asak</i> (Forssk.) Willd. SUDAN: Kassas & Obeid E 349, 12.12.1967 (KHU).
3	<i>A. ataxacantha</i> DC. SUDAN: Garsila. Wickens 1604 (KHU).
4	<i>A. drepanolobium</i> Harms. ex Sjostedt. SUDAN: Khor Yabous. Igbal 129, 18.3.1976 (KHU).
5	<i>A. ehrenbergiana</i> Hayne. SUDAN: Khartoum. Kassas 19, 10.8.1953 (KHU).
6	<i>A. etbica</i> Schweinf. SUDAN: Erkwit. Kassas 221, 7.12.1966 (KHU).
7	<i>A. gerrardi</i> Benth. SUDAN: Zalingei. Wickens 2754 (KHU).
8	<i>A. laeta</i> (R. Br. ex Benth.) Benth. SUDAN: Wad El-Nile. M. Osman 21.9.1966 (KHU).
9	<i>A. mellifera</i> (Vahl) Benth. SUDAN: Khor Arbaat. Kassas, 20.2.1954 (KHU).
10	<i>A. nilotica</i> (L.) Willd. ex Del., subsp <i>adansonii</i> (Guill. & Perr.) Brenan. SUDAN: Kibi. Wickens 1852 (KHU).
11	<i>A. nilotica</i> subsp <i>nilotica</i> (L.) Del. SUDAN: Wadi Halfa. Bror Pettersson & Teuvo Ahti 16269 (KHU).
12	<i>A. nilotica</i> subsp <i>tomentosa</i> (Benth.) Brenan. SUDAN: Kassas <i>et al.</i> E. 137 (KHU).
13	<i>A. nubica</i> Benth. SUDAN: W. Omdurman. Kassas 9, 27.7.1953 (KHU).
14	<i>A. polycantha</i> Willd. subsp <i>camplacantha</i> (Hochst. ex A.Rich.) Brenan. SUDAN: Wadi Nimr. Kassas <i>et al.</i> 360-8, 12.12.1965 (KHU).
15	<i>A. senegal</i> (L.) Willd. var. <i>senegal</i> Brenan. SUDAN: Zalingei. Wickens 1804 (KHU).
16	<i>A. seyal</i> Del. var. <i>fistula</i> (Schweinf.) Oliv. SUDAN: Tuzi (South Blue Nile). M. Osman, 20.9.1966 (KHU).
17	<i>A. seyal</i> Del. var. <i>seyal</i> (Yellowish bark). SUDAN: Wadi Halfa. Bror Pettersson & Teuvo Ahti 16274, 3.10.1962 (KHU).
18	<i>A. seyal</i> Del. var. <i>seyal</i> (Red bark). SUDAN: Gadarief. Kassas <i>et al.</i> E 548 (KHU).
19	<i>A. sieberiana</i> DC. var. <i>vermoesenii</i> (De Wild.) Deay & Brenan. SUDAN: Beldong. Wickens 2159 (KHU).
20	<i>A. sieberiana</i> DC. var. <i>sieberiana</i> . SUDAN: Gallabat. Kassas <i>et al.</i> E 669 (KHU).
21	<i>A. sieberiana</i> DC. var. <i>villosa</i> A. Chev. SUDAN: Zalingei. Wickens 1568 (KHU).
22	<i>A. tortilis</i> (Forssk.) Hayne subsp <i>raddiana</i> (Sav.) Brenan. SUDAN: Desert W. of Omdurman. A/salam, 5.9.1965 (KHU).
23	<i>A. tortilis</i> (Forssk.) Hayne subsp <i>tortilis</i> Brenan. SUDAN: Wadi Seidna Enclosure. R. Halwagy 512, 5.4.1960 (KHU).

The specimens were coated with gold using Sputter Coater and examined with GEOL JSM-7200 Scanning Electron Microscope (SEM) using an accelerating voltage of 25 kV and 8 mm working distance. Micrographs of the leaflets for each species were taken using (X200) and (X1000) magnifications. The terminology used is that of Barthlott *et al.* [9], Culter [15] and Weiller *et al.* [16].

Results

a) Inter-specific variations

Amorphous to weakly undulated wax surfaces and two structured (sculptured) wax types were encountered to exist on the adaxial surfaces of the leaflets of the *Acacia* species examined. Amorphous to weakly undulated (granular) wax surfaces (Fig. 1) were observed in *A. gerrardi* and *A. ehrenbergiana*. Structured (sculptured) wax deposits occurred either in a rosette form of platelets (Fig. 2 A) as in *A. etbica*, *A. drepanolobium*, *A. nilotica* (and the subspecies), *A. nubica*, *A. seyal* (and the varieties), *A. sieberiana* (and the varieties), *A. tortilis* (and the subspecies), *A. polycantha* and *A. albida*, or the platelets coalesce to form ridge-like structures (Fig. 3 C) as in *A. laeta*, *A. ataxacantha*, *A. asak*, *A. mellifera* and *A. senegal*.

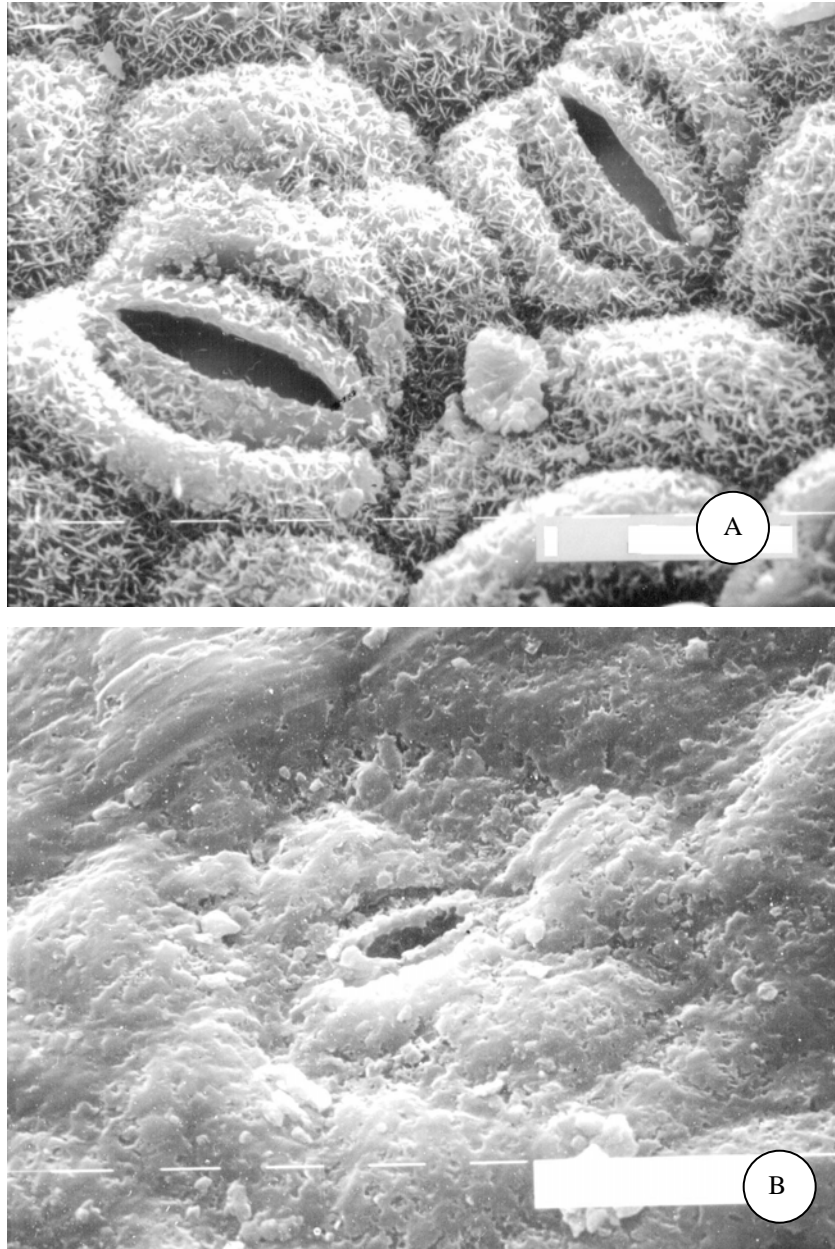


Fig. 1. Scanning electron micrographs of the adaxial surfaces of the leaflets of African acacias. (A) *A. gerrardi* showing amorphous wax surface. (Bar lines = 50 μm). (B) *Acacia ehrenbergiana* showing weakly undulating (granular) wax surfaces (Bar lines = 50 μm).

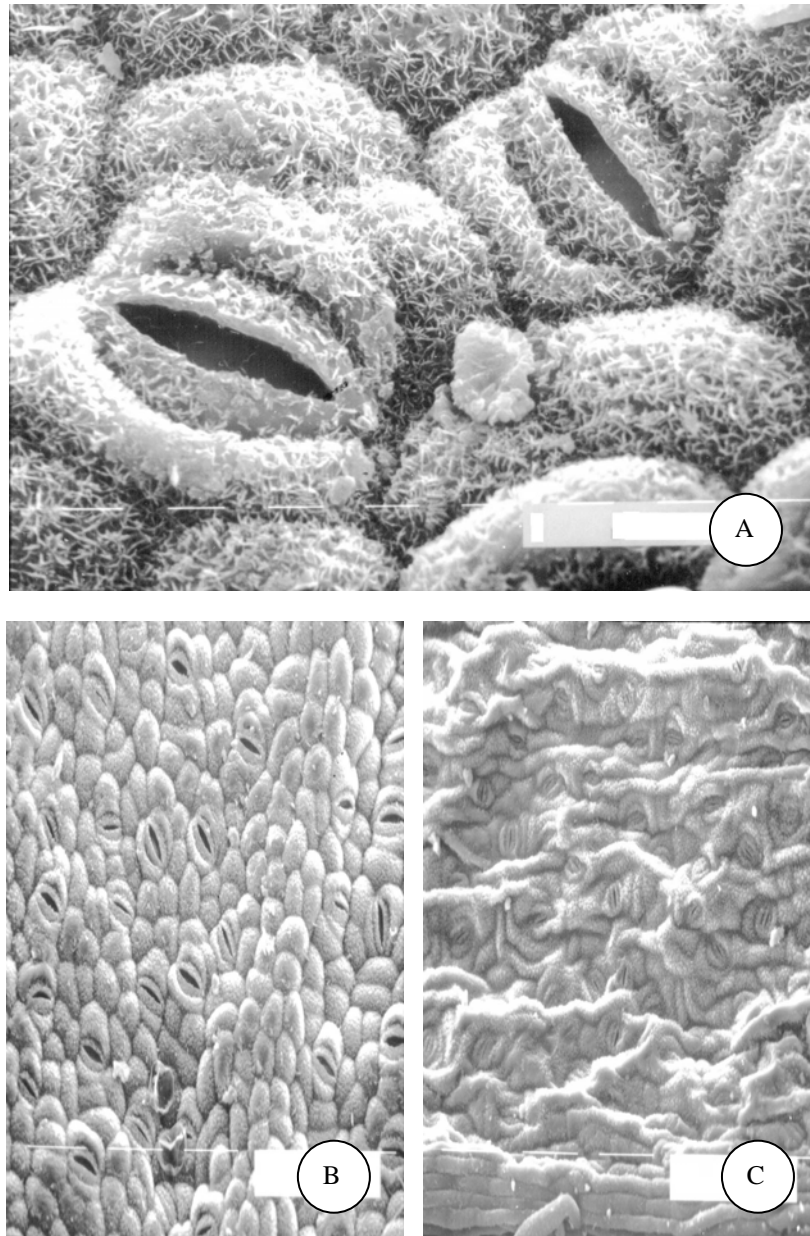


Fig. 2. Scanning electron micrographs of the adaxial surfaces of the leaflets of African acacias. (A-B) *Acacia etbica* showing wax deposits in a rosette form of platelets, and epidermal cells \pm isodiametric (regular) in shape (in A Bar lines = 50 μm , in B Bar lines = 5 μm). (C) *A. drepanolobium* showing epidermal cells irregular in shape (Bar lines = 5 μm)

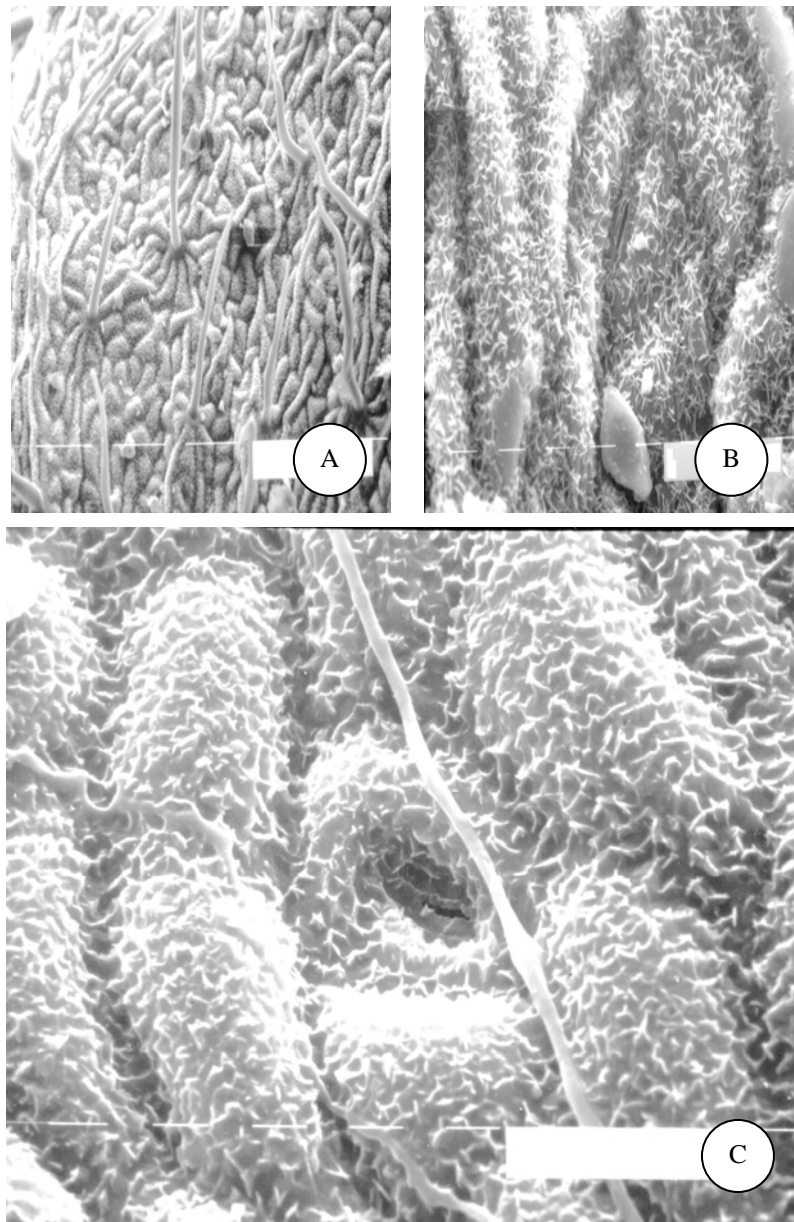


Fig. 3. Scanning electron micrographs of the adaxial surfaces of the leaflets of African acacias. (A-B) *Acacia albida* showing trichomes distributed all over the surface and epidermal cells irregular in shape (in A Bar lines = 5 μm , in B Bar lines = 50 μm). (C) *A. laeta* showing wax deposits in the form of ridge-like structures (Bar lines = 50 μm).

Additional features observed on the surfaces of the leaflets include the shape of the epidermal cells and the distribution of the trichomes. The epidermal cells were found to be \pm iso-diametric (regular) (Fig. 2B) only in *A. etbiaca*, and irregular in shape (Fig. 2C) in the rest of the species. *A. albida* within the species examined represents the only species with trichomes distributed all over the adaxial surfaces of the leaflets.

b) Leaflet-types:

Within the species examined, five leaflet-types were recognized. These types differ in the epicuticular wax deposited, shape of epidermal cells and the distribution of trichomes on the surface of leaflets.

1. *A. gerrardi* – type (Fig. 1 A-B):

Amorphous to weakly undulated (granular) epidermal surfaces, epidermal cells convex and irregular in shape, and without trichomes. This type existed in *A. gerrardi* and *A. ehrenbergiana*.

2. *A. etbica* – type (Fig. 2 A-B):

Epicuticular wax deposits in a rosette form of platelets, epidermal cells convex to strongly domed and \pm iso-diametric (regular) in shape, and without trichomes. This type existed in *A. etbica* only.

3. *A. drepanolobium* – type (Fig. 2 C):

Epicuticular wax deposits in a rosette form of platelets, epidermal cells convex and irregular in shape, and without trichomes. This type existed in *A. drepanolobium*, *A. nilotica* (and the subspecies), *A. nubica*, *A. seyal* (and the varieties), *A. sieberiana* (and the varieties), *A. tortilis* (and the subspecies), and *A. polycantha*

4. *A. albida* – type (Fig. 3 A-B):

Epicuticular wax deposits in a rosette form of platelets, epidermal cells convex and irregular in shape and with trichomes distributed all over the adaxial surfaces. This type existed in *A. albida* only.

5. *A. laeta* – type (Fig. 3 C):

Epicuticular wax deposits in the form of ridge-like structures, epidermal cells convex and irregular in shape, and without trichomes. This type existed in *A. laeta*, *A. ataxacantha*, *A. asak*, *A. mellifera* and *A. senegal*.

Discussion and Conclusions

Numerous attempts have been made to divide the African acacias into different taxa. These divisions were previously made on the basis of morphological characters like: the nature of spines, number of leaflets, shape of flowers, type of inflorescence, pollen characters, fruit morphology, etc. Such attempts resulted in a bewildering number of different taxa indicating that the genus *Acacia* is in need of more broad-based comparative studies.

The present study revealed interesting inter-specific variations in the epicuticular wax deposits on the adaxial surfaces of the African *Acacia* leaflets that are of significance for identification.

Three different epicuticular wax deposits were recognized in the material examined. Amorphous to weakly undulated wax was observed in two species, rosette form of wax platelets were observed in 17 species and ridge-like wax platelets in five species. These intra-specific differences demonstrate the existence of mixed epicuticular wax deposits in the genus *Acacia*.

Mixed epicuticular wax deposits also occur in the genera *Aloe* [15], *Bupleurum* [17], *Eucalyptus* [18-19], *Ficus* [10], *Gethyllis* [20], *Viscum* [21], etc. Such differences may demonstrate that the present status of these genera is in need of taxonomic revision.

Previous classification of the African members of the genus *Acacia* [3-5] divided the acacias into two groups, namely; *Gummiferae* (= *Acacia*) and *Vulgares* (= *Aculeiferum* = *Senegalia*). The first group (*Gummiferae*) includes those species with straight axillary thorns (*A. gerrardi*, *A. ehrenbergiana*, *A. etbica*, *A. drepanolobium*, *A. nilotica* (and the subspecies), *A. nubica*, *A. seyal* (and the varieties), *A. sieberiana* (and the varieties), *A. tortilis* (and the subspecies), and *A. polycantha* and *A. albida*). The second group (*Vulgares*) includes those species with prickly recurved, claw-shaped thorns (*A. laeta*, *A. ataxacantha*, *A. asak*, *A. mellifera* and *A. Senegal*). According to the present study, the first group (*Gummiferae*) comprises *A. albida*-type, *A. etbica* – type, *A. drepanolobium* – type and *A. gerrardi* – type which is characterized by the presence of rosette forms of wax platelets and amorphous to weakly undulating (granular) waxes, whereas the second group comprises *A. laeta* – type only which is characterized by the presence of ridge-like wax platelets. The present study highlighted that the first group (*Gummiferae*), comprises at least four leaflet-types, and is the group which needs further segregation into at least four subgroups.

A. albida which is the only representative of *A. albida* - type has already been segregated from the genus *Acacia* and transferred into a separate genus *Faidherbia* (Del.) A. Chev. The peculiar characteristics of *A. albida* as observed in the present study were also endorsed by previous findings from pollen morphology [22], chloroplast DNA sequence [23-24], biochemistry [25], phytochemistry [26], chromosomal number [27], etc. The present study supports the arguments that a separate taxon should be created to accommodate *A. albida*, but not the establishment of a separate genus for this species due to its close affinity in wax morphology.

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كلية المعلمين بمحافظة الرس، ص ب ٥٣ الرس،
المملكة العربية السعودية

(قدم للنشر في ٢٣/١٠/١٤٢٧هـ؛ وقبل للنشر في ١٤/٢/١٤٢٨هـ)

. تم فحص وريقات لـ ٢٦ نبات أفريقي تنتمي إلى جنس الأكاشيا باستعمال المجهر الإلكتروني الماسح (SEM) في محاولة لتحديد بعض من الصفات التي لها أهمية تصنيفية. تم التعرف على ثلاثة أشكال من ترسيبات الشمع المتواجد فوق البشرة، وكذلك خمسة أنواع وريقات في جنس الأكاشيا. تختلف هذه الأنواع من حيث ترسيبات الشمع وشكل خلايا البشرة وتوزيع الأوبار على سطح الوريقات. تتفق أنواع هذه الوريقات مع التقسيمات الأساسية السابقة للأكاشيا، كما تم اقتراح تقسيمات فرعية على ضوء الاختلافات النوعية في أشكال الشمع. أبرزت الدراسة أن ترسيبات الشمع المتواجدة فوق بشرة السطوح البطنية لنباتات الأكاشيا مقرونة مع صفات أخرى يمكن أن تُستعمل كأداة جيدة لتحديد الفئات التصنيفية لنباتات الأكاشيا.

