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The Significance of Cuticular Features, Petiole Anatomy and SDS-PAGE in the Taxonomy of the Lauraceae

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Abstract: SDS-PAGE of seed protein, petiole anatomy and leaf cuticular features were used to re-assess the taxonomic relationships between eight taxa presenting four genera of the Lauraceae, viz. *Apollonias* Nees (one species), *Cinnamomum* Schaeff (three species), *Laurus* L. (two species) and *Persea* Mill. (one species with two varieties). The data obtained were analyzed by Ntsys-pc program package using the UPGMA clustering method. The produced dendrograms were discussed. SDS-PAGE analysis showed a close relationship between *Apollonias* and *Persea*. Domatia and certain unique features were recorded in the leaf epidermis of *Apollonias*. Considerable variations were evident between the two varieties of *Persea* and a re-evaluation of their status was suggested. The validity of SDS-PAGE of seed protein criteria in the Lauraceae was confirmed.

Key words: *Apollonias*, *Cinnamomum*, cuticles, electrophoresis, Lauraceae, petiole

Introduction

The Lauraceae (50 genera and 2500 species *sensu*, Mabberiey, 1997) is a generalized monophyletic family dating back at least to the mid Cretaceous (Rohwer, 2000). However, the relationships within the family are still poorly understood; generic delimitation is often problematic and identification of genera and species is extremely difficult without flowering and fruiting materials (Rohwer, 2000). Several supra-generic classifications of Lauraceae were put, using different criteria; macro morphological and floristic (Hutchinson, 1964), wood and bark anatomy (Richter, 1981), a combination of anatomy and morphology (Rohwer, 1993) and molecular aspects (Rohwer, 2000). Yet further research is still needed to achieve a conclusive result (Rohwer, 2000). Many authors used cuticular studies in the Lauraceae in an attempt to differentiate between species or to clarify the relations between them (Hill, 1986). However, it was Christophel *et al.* (1996) who drew the attention to the importance of cuticular features in the taxonomy of the Lauraceae.

Furthermore, they stressed on the fact that the family makes an ideal candidate for a global study based on leaf cuticles. Also, petiolar anatomy has served as an aid in the identification and the delimitation of some taxa in the Lauraceae (Balasubramanian *et al.*, 1993 and several others). On the other hand, seed proteins are highly stable, being unaffected by environmental conditions (Harbome and Turner, 1984). Thus, electrophoretic patterns of total seed protein (protein profiles) as revealed in the presence of Sodium Dodecyi Sulfate(SDS- PAGE) have provided a valid source of taxonomic relationships at the generic and specific level in different families (Badr *et al.*, 2000).

The present study deals with the use of SDS- PAGE (seed storage protein profiles), petiolar anatomy and leaf cuticular aspects to provide more information about the taxonomic relationships of eight taxa of Lauraceae (four genera including seven species and one variety) representing the tribes Laureae and Perseae (*sensu*, Rohwer, 1993). The produced characters are analyzed by the Ntsys-pc program package, using the UPGMA clustering method. The phenograms produced are discussed in the light of the current systems of classification.

Materials and Methods

The examined taxa and their sources are listed in Table I. The voucher specimens are kept at the herbarium of Department of Biological Sciences and Geology, Faculty of Education, Ain Shams University, Cairo, Egypt. Epidermal peels for cuticle examination were prepared as shown by Christophel and Rowett (1996) at the Faculty of Science, Ain Shams University. The anatomical investigation was achieved through transverse sections of the leaf petiole by a hand microtome at 20 μ and stained with Safranin and Light green at the Faculty of Science, Ain Shams University.

The epidermal peels and petiole sections were photographed using a Carl -Zeiss photo-microscope III. Magnifications are expressed as X. Description and terminology presented by Christophel and Rowett (1996) was followed to describe the cuticular aspects. For SDS-PAGE electrophoresis, 0.1 gm of seeds were mixed with an equal weight of pure, clean, sterile fine sand and powdered using mortar and pestle. Extraction of proteins was carried out using Tris-HCl (8.0) in the presence of 2-mercaptoethanol (under reducing condition). The powder was homogenized with 1 ml of the buffer for 2 hr at 20 °C. SDS- PAGE was carried out in 12.5 % acrylamide gels in Tris-glycine running buffer (pH 8.3) at 150 V for 3 hr using a low molecular weight protein of Sigma as a marker. Gel were then stained in Comassie brilliant blue R-250 for 30 min., destained, photographed and molecular weight values for subunits were determined by comparison with standard proteins as described by Matta *et al.* (1981). Analysis was carried out using pro-analyzer version 2.0.

For data analysis, the total number of recorded characters (76) in each taxon, were scored, combined together in two sets of data and coded for creating the data matrix of computation: (a) cuticular features and anatomical aspects of petiole, (b) SDS-PAGE criteria, © All characters combined. The presence or absence of each — different characters was treated as a binary character in a data matrix i.e. coded 1 and 0 respectively.

The relationships between the studied taxa, expressed by average taxonomic distance (dissimilarity), have been demonstrated as phenograms, based on the analysis of the recorded characters using the Ntsys program package for IBM-pc as described by Rohlf (1993).

Results

Leaf cuticular features of the studied taxa; Terminology is taken after Wilkinson (1979) and Christophel and Rowett (1996b) and taxa numbered 1 - 8 are presented in Plate 1.

Upper epidermis:

a) Overall cell shape: Angular in 2 & 3 sinuate in 4, 5 & 6 and angular to rounded in 1,7&8.

Table 1: Taxa studied, their sources and geographical distribution.

Taxon	Tribe		Source	Common name	Geographical distribution
	Kostermans (1973)	Rohwer (1993)			
Apollonias barjujana (Cav.) Bornm. = *A. canariensis* (Willd.) Nees	Perseae	Perseae	J.B.C.	Barbusano	Macaronesia
Cinnamomum camphora (L) J.presl = *Laurus camphora* L.	Cinnamomeae	Perseae	O.B.G. Q.B.G. Cairo	Camphor plant	China and Japan
Cinnamomum glanduliferum (Wallich) Ness = *Laurus glandulifera* Wallich	Cinnamomeae	Perseae	O.B.G.	-	Central Himalayas
Cinnamomum verum J. Presl = *C. zeylanicum* Blume = *Laurus cinnamomum* L.	Cinnamomeae	Perseae	O.B.G B.G.F.Sc. Cairo	Cinnamon	India, Malaya and widely cultivated in tropics
Laurus azorica (Seub) Franco =*L. canariensis* Webb and Berth.	Laureae	Laureae	J.B.C. B.G.F.Sc. Alex	Canary Island Laurel	Macaronesia and Morocco (small population)
Laurus nobilis L.	Laureae	Laureae	O.B.G. B.G.F.Sc. Cairo	Bay laurel and Sweet bay	Mediterranean region
Persea americana var. americana Miller. = *P. leiogyna* S.F. Blake	Laureae	Laureae	B.G.F.E. Cairo	West Indian Avocado	Tropical America
Persea americana var. Drymifolia S.F. Blake = *P. drymifolia* Schlecht and cham	Laureae	Laureae	B.G.F.Sc. Avocado	Maxican Avocado	Tropical America

J.B.C. = Jardin Botanico Canario "Viera Y Clavijo" Las Palmas - Gran Canaria
O.B.G. = Orman Botanic Garden, Giza-Egypt.
B.G.F.Sc.Cairo = Botanical Garden of Faculty of Science, Ain Shams University, Cairo-Egypt.
B.G.F.Sc. Alex = Botanical Garden of Faculty of Science, Ain Shams Unversity, Alexandria-Egypt.
B.G.F.E. Cairo = Botanical Garden of Faculty of Science, Ain Shams Unversity, Cairo-Egypt.
Q.B.G. = Qubba Botanical Garden, Cairo-Egypt.

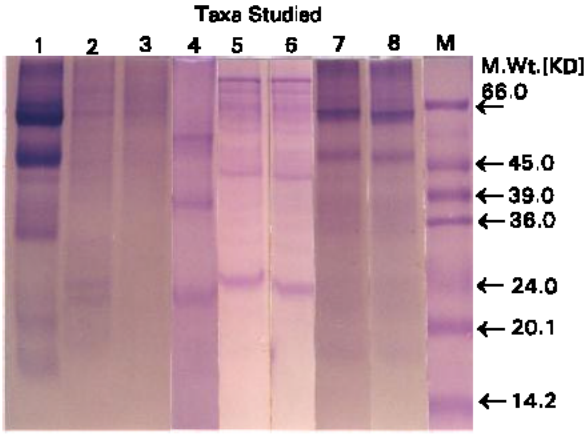

Fig. 1: Electrophoretic-banding profiles of seed proteins extracted in Tris-HCl buffer of the studied taxa of the Lauraceae.

b) Anticlinal walls: (I- thickness degree): very thick in 1 and thick in 2 - 8. **(ii- pattern of thickening):** buttressed in 4, 5 & 6 and uniform in 1, 2, 3, 7 & 8.

c) Periclinal walls: (texture): finely granular in 1, 2, 4, 5 & 6, granular punctate in 3 & 7 and granular with glandular bodies prominent in 8.

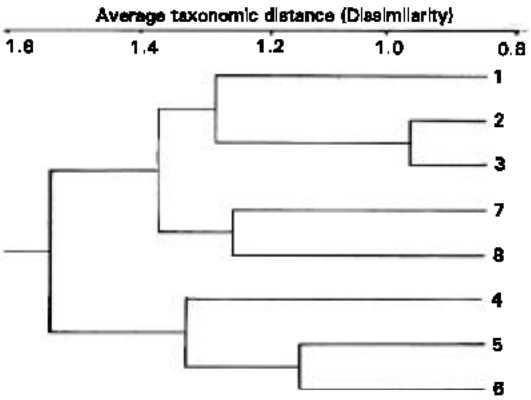

Fig. 2: UPGMA- phonogram based on coding of 56 attributes obtained from cuticular features and petiole anatomy.

Lower epidermis:

a) Overall cell shape: irregular to rounded in 1, 2, 3, 7 & 8 and sinuate in 4, 5 & 6.

b) Anticlinal walls: (I- shape): undulate to straight in 1, straight

in *1*, 3, 7 & 8 and highly sinuate in 4, 5 & 6. **(ii- degree of thickness):** very thick in 5 & 6, thick in 1, 2, 3, 7 & 8 and varying in 4. **(iii- pattern of thickening):** irregular in 2, 3, 5 & 7, buttressed in 4 & 6, uniform in 8 and slightly buttressed in 1.

c) Periclinal walls: (texture): granular in 1 -8.

d) Stomatal complex: (I- subsidiary cells - anticlinal walls): - degree of thickness: thin in 1 & 4, slightly thick in *2, 3, 7 &* 8 and thick in 5 & 6. **Pattern of thickness:** uniform in 1 & 4, slightly beaded in 2, 7 & 8, buttressed in 3 and slightly buttressed in 5 & 6. **(ii-periclinal walls): - thickness:** variable, with prominent ledge around margin of stomatal scales in 1 and granulate in 2 - 8. **(iii-stomatal scales): - shape:** butterfly shaped in 1 & 4 and narrow, crescent shaped in 2, 3, 5, 6,7&8.

e) Special features: (I-trichomes): present in 7 & 8 and generally absent or not prominent in 1 - 6. **Shape:** medium sized, pointed and unbranched in 8 and stout, long, pointed and unbranched in 7. **(ii-domatia:** present in 1 and absent in 2 - 8. **(iii-)** in all the studied taxa, epidermal cells tend to be somewhat elongated over the veins.

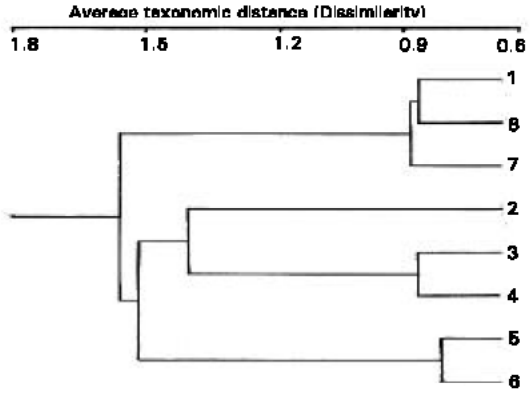

Fig. 3: UPGMA-phenogram based on coding of 20 attributes obtained from SDS-PAGE profiles of seed proteins extracted in Tris-HCl buffer.

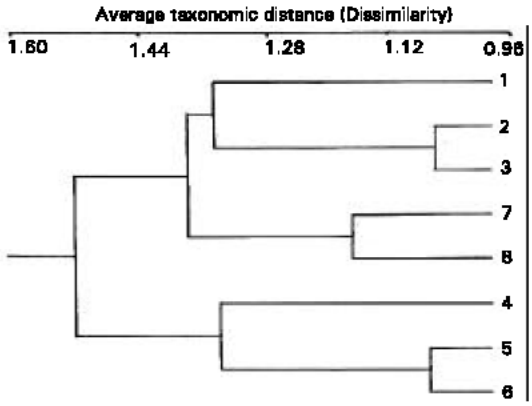

Fig. 4: UPGMA- phenogram based on coding of 76 attributes obtained from cuticular features & petiole anatomy and SDS-PAGE profiles of seed proteins

Petiole anatomical aspects: Terminology is taken after Steam (1992) and taxa are numbered 1 - 8 as presented in Plate II. **Shape in cross-section:** Reniform in 4, 5 & 8, napiform in *7*, globose in 6, irregular in 2 and cuneiform (wedge shaped) in 1 & 3.

Adaxial surface: Concave (grooved) in 4, 5 & 8, convex in 1 - 3 and straight in 6 & 7.

Trichomes: (I- number): varying in 5 - 7 and absent in 1 - 4. **(ii- shape):** short, pointed and unbranched in 5 - 7. **(iii- location):** all over the petiole in 7 and adaxial surface only in 5 & 6.

Epidermal cells: rounded to barrel-shaped in 1 - 8.

Hypodermis: multilayered collenchyma in 1-8.
Oil cells: transparent rounded cells, scattered in the ground tissue in 1 - 8.

Pigmented cells: (location): Scattered in the ground tissue in 6 & 7 and located near the outer layers of the petiole in 1, 2, 3, 4, 5 & 8.

Vascular strand: (I- shape): crescent in 1 - **8. (ii- location):** center in 1 - 8. **(iii- number of bundles):** 15 - 20 in 1 - 8.

SDS-PAGE of seed protein criteria: In total, 20 protein bands were revealed in the electropherogram of the eight taxa studied in the Lauraceae using Tris-HCl (pH 8) as extraction buffer (Fig. 1). The highest band number (11) was scored in the banding profile of *C. camphora*, while the lowest number (6) was found in *C. verum*. Seven bands were observed in two species *of Laurus* and the two species *of Persea*.
The recorded bands have molecular weight ranging between 16.5 KD and 72.8 KD. Bands having molecular weights of 72.8, 60.0, 56.0, 45.0, 41.0 and 27.0 KD are common in the taxa studied

Discussion

Although the monophyly of the Lauraceae s.l. is unquestionable and strongly supported by both morphological and molecular aspects (Rohwer, 2000); yet a more conclusive delimitation of genera and species and/or deducing the inter-relationships between them is still far from reach and cannot be achieved except by the incorporation of new data sets in this process (Rohwer, 2000).
In present study, cuticular features of leaves and petiolar anatomy supported the monophyly of the family. In all studied taxa leaves were hypostomatic, cell patterns of the upper epidermis often differed from the lower ones. Two main cell types were observed (angular and sinuate), the cells often elongating over the veins (Plate I). The same results were observed by Christophel and Rowett (1996) on the Australian species of the family. The most prominent cuticular aspect observed was the stomatal complex, for although the stomata are parasytic, subsidiary cells took the characteristic shape of the guard cells observed in other families, while the true guard cells were sunken; cuticular ledges often protruded from the inner edges of the guard cells and/or subsidiary cells and took various shapes (Plate I). This feature seems to be a synamorphy that unites the Lauraceae worldwide in both fossil and extinct taxa (Christophel and Rowett, 1996).
Apollonias barbujana possessed certain unique features of its own as the very thick anticlinal walls of the upper epidermis cells, and the presence of domatia on the lower epidermis (Plate I). It is worth mentioning that *Apollonias barbujana* constituting (together with few Lauraceous genera) a relict plant formation in Macaronesia (Press and Short, 1994).

As to the petiolar anatomy (Plate II), the vascular strand took a crescent shape in all the studied taxa. Transparent oil cells and pigmented cells were seen scattered in the ground tissue. The same phenomena were observed in other worldwide genera of the Lauraceae (Metcaife and Chalk, 1950).

The phenogram constructed according to the analysis of 56 characters of leaf cuticular features and petiole anatomy (Fig. 2) showed a close similarity between *Cinnamomum camphora* (2) and *C. glanduliferum* (3), both taxa clustered at the dissimilarity level of 0.93. This was due to their angular epidermal cells, with generally straight and uniform anticlinal walls, narrow and crescent shaped cuticular ledges (Plate I). Vegetative macromorphology seems to support such a similarity between the two taxa, as both possess scaly dormant buds on the main stem and branches. These buds often produce recurrent flushes of epicormic shoots (angiosperm phenomenon described by Kozlowski and Pallardy (1997). However, *C. verum* (4) showed a considerable divergence from the former two species *of Cinnamomum*. It clustered within the second group along with *Laurus* species (5 & 6) at the dissimilarity level of 1.30 (Fig. 2), mainly due to the possessing of *C. verum* and *Laurus* species to sinuate epidermal cells with a buttressed thickening pattern (Plate I).

The variations encountered between *Cinnamomum* species either from cuticular or petiole anatomical aspects may suggest the paraphyly of this genus (350 species *sensu* Mabberiey, 1997), as Christophel *et al.* (1996) recorded three different cuticular patterns in Asian, Australian and South American species of *Cinnamomum*. Finally, the phenogram showed that considerable variations were prominent between the two varieties of *Persea* (7 & 8); both taxa clustered at the dissimilarity level of (1.20). This was mainly due to the presence of prominent glandular bodies on the upper epidermis of *P. americana* var. drymifolia and it possessed a grooved petiole devoid of trichomes, while the other variety possessed a non-grooved petiole, napiform in cross-section and covered all over by trichomes. The variations encountered between the two varieties are supported by macro morphological aspects (Bailey, 1949) and a re-evaluation of their status is suggested here.

The phenogram constructed according to the analysis of 20 characters of SDS-PAGE of seed proteins, showed a considerable similarity between *Apollonias barbujana* (1) and both varieties of *Persea americana (7* & 8). The three taxa clustered at the dissimilarity level of 0.83 (Fig. 3).

This result agreed with Rohwer (1993), who stressed on the close relationship of both *Apollonias* and *Persea*. Moreover, Rohwer (2000) stated that both genera constitute (along with several others) a real phylogenetic alliance. The grouping of *Cinnamomum* species along with *Laurus* species in the second major group and their clustering together at a dissimilarity level of 1.50, while separating from the first group (including *Apollonias* and *Persea)* at the dissimilarity level of 1.54 (Fig. 3), agreed with cladistic treatment of the Lauraceae presented by Rohwer (2000) based on molecular evidence *(mat* K-sequences), who showed that *Cinnamomum* may be nearer to *Laurus* than to *Persea* and *Apollonias* as was traditionally accepted.

Finally, the close similarity observed between the two species *of Laurus* (clustering together at a dissimilarity level of 0.75 gives extra support to Rohwer (1993b) suggestion that the two species of *Laurus* might better be treated as two subspecies, and agree with Loutfy (2000), who showed that the variation between the two species were merely quantitative.

The last phenogram based on all the studied characters (76) showed clearly the divergence of *Cinnamomum* species (Fig. 4). The first two species grouped with *Apollonias* and *Persea* while *C. verum* grouped with *Laurus* species. This result agreed with Van der Werff and Richter (1996), who stated that *Cinnamomum* appears to be a transitional genus, having affinities with both tribes Laureae and Perseeae with closer affinities to the latter.

Finally, the data obtained from SDS- PAGE of seed protein of the studied taxa showed a high consistency with the evidence obtained by molecular criteria (Rohwer, 2000) and so the validity of SDS-PAGE of seed protein criteria in the taxonomy of Lauraceae could be documented. Data obtained from cuticular features and petiole anatomy were mainly useful in deducing the relations between taxa belonging to the same genus, and/or closely related genera, while in distantly related ones, further studies on more cosmopolitan material of the Lauraceae is still needed before a comprehensive conclusion can be achieved. This fact was explored earlier by Graybeal (1998), who showed that more accuracy and a better resolution of the produced phenogram was much higher, if the constant characters were distributed across a

larger number of taxa; a difficult task in the Lauraceae in particular as shown by Rohwer (2000) due to the fact that many taxa in the family are still unknown to man, new genera and species are still discovered and identified from different tropical and uninhabited places throughout the world.

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