ISSN:2319-2607 IISUniv.J.Sc.Tech. Vol.2(1),43-48 (2013)
RESEARCH ARTICLE

Epidermal and Venation Studies in *Carissa carandas L.* and *Alstonia scholaris R. Br.*

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Abstract

The present investigation is based on the microanatomical characteristics including epidermal cells, stomata, trichomes, major and minor venation i.e. vein- islets and free vein- ending in *Carissa carandas* and *Alstonia scholaris* collected from various localities of Jaipur District. A detailed study of the micro-anatomical characteristics of two species belonging to the family Apocynaceae was carried out to utilize the variations of different features like stomatal index and frequency,venation patten , presence or absence of trichomes etc. in the delimitation of taxa at different taxonomic hierarchy.

Keywords

Alstonia scholaris, Apocynaceae, *Carissa carandus,* Microanatomical Characteristics

Introduction

Epidermal morphology of the leaf has been extensively used in emphasizing the relationship and ontogeny of different taxa. Chandra *et al* (1969) emphasized the significance of epidermal morphology and arrangement as a useful tool in phylogenetic and taxonomic consideration. The importance of epidermal characters of leaves in angiosperms have been reviewed by Stace (1984). Several authors have shown the importance of epidermal features in recognition of different taxa of plants (Hagerup, 1953; Borril, 1961; Tomblinson, 1979; Nyawuame and Gill, 1990, 1991; Ogundipe and Akinrinlade, 1999; Praveen *et al*, 2000).

Indeed the Angiospermous foliar epidermis is an easily studied character that shows sufficient diversity of details (Kannabiran and Krishnamurthy, 1972).The size, distribution and frequency of stomata have been reported to be specific to the taxa below the rank of family and the stomatal characters are very significant parameters in angiosperm taxonomy and phylogeny (De Bary, 1884; Metcalf and Chalk,1950, 1979; Karatela and Gill, 1986; Mukherjee *et al*, 2000).

The family Apocynaceae is also known as Dogbane or Oleander family. As currently recognized the family has 424 genera in five subfamilies: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae. It includes trees, shrubs, herbs usually with milky sap. The classification of Apocynaceae followed the Bentham and Hooker's system. The last three subfamilies have traditionally been placed in a separate family Asclepiadaceae that belongs to the same order Gentianales as does Apocyanaceae but this division in to two families has become more and more unsound with mounting evidence (Endress and Bruyans, 2000). However, traditionally the two families has been studied separately with the result that our understanding of the species diversity in the two groups is quite uneven. Many reports have been published on the Apocynaceae *sensu stricto* (i.e. subfamilies Rauvolfioideae and Apocynoideae) by Leeuwenberg (1985, 1991, 1998); Rudjiman ,1986; Sidiyasa,1998 and Hendrian and Middleton ,1990 . Middleton has been credited with the most comprehensive work on the family Apocyanaceae (1993, 1995a, 1995b, 1996a, 1996b, 1996c, 1997, 1999, 2000).

The species are distributed mainly in tropical rainforest and most are from the tropics and subtropics but some come from tropical dry, xeric environments. There are also some perennial herbs from temperate zones.The leaves are simple, usually opposite and decussate, or whorled; lacking stipules. Flowers are usually showy, radially symmetrical (actinomorphic), aggregated in cymose or racemose inflorescences (rarely fasciculate or solitary). They are perfect (bisexual), with a synsepalous, 5- lobed calyx. Inflorescences are terminal or axillary. The stamens are inserted on the inside of the corolla tube. The ovary is usually superior.The fruit is drupe, a berry, a capsule or a follicle. Many plants of this family have economic and medicinal values for example, as source of inferior rubber,

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as source of fiber, as venom for arrow tips, as uterine contraction agents, as source of alkaloids etc.

Apocyanaceae is a very large family of Angiosperm and has been anatomically studied by different workers from different point of view including foliar epidermis also. (Kapoor *et al*,1969; Sharma *et al*,1970 and Chandra *et al*,*1972*). As such, the present paper reveals the leaf epidermal micro-morphology of two taxa of family Apocyanaceae: *Carissa carandas* and *Alstonia scholaris* .

Materials and Method

Collection of Plant materials

Polythene bags (18" X 12") were used for collecting the plants and these were found to be very convenient and efficient than the conventional metallic vasculum.

For the present foliar studies only fresh materials were used. The leaves of the selected taxa were collected from various localities in Jaipur city .In addition to fresh specimens, the materials were also fixed in formalin acetic acid alcohol (FAA). This was done for verification of any desired features at a later stage .

Preparation of peeling for epidermal studies

Foliar micro- morphological studies were done using fresh specimens. For fresh leaves of *Cassia carandas* , epidermal peeling were taken by direct peel method, stained in safranin and mounted in glycerine. Peeling from leaves of *Alstonia scholaris* were obtained by scraping off all the unwanted tissue using a sharp edged scalpel and forceps & mounted in pure glycerine for study. In case where it was not possible to remove the epidermis by forceps method, the following methods were employed.

a. Middle portion of the lamina was kept in 10-30% of HNO_3 solution for 12-24 hours. The treatment was effective in separating the epidermal layers from the mesophyll cells. After a gentle wash in water the pieces of separated epidermal layers of both side were removed from the mesophyll tissue with the help of needle and scalpel.

b. The triple acid treatment method was employed (Ramayya and Vanaja, 1979) for obtaining the epidermis of comparatively hard textured leaves of *Alstonia*. In this method, a mixture of 10% each of chromic acid, glacial acetic acid and nitric acid in equal proportion was used. The leaves were boiled for 10-20 minutes in this mixture and then left for 20 minutes to cool down. The material was washed with water. This treatment separated epidermis from the mesophyll tissue.

The epidermal pieces obtained by the above method were stained in safranin and were used for studying stomata, trichomes and other epidermal characteristics.

Clearing Technique

Mature leaves were collected and fixed in FAA to remove chlorophyll. Small pieces from the middle portion of lamina were used for clearing purpose. For the study of vein- islets and vein- endings, pieces of lamina were placed in I5% KOH solution and later transferred to a saturated solution of 250% Chloral hydrate ($CC_{13}CH(OH)_2$) which was heated till the pieces become transparent. Cleared leaf pieces were then washed twice in distilled water and stained with safranin.

Calculation

All measurements were an average of 50 readings. Stomatal index and frequencies were calculated by the method suggested by Salisbury (1927, 1932).

Stomatal Index

S.I.= (S/S+E) X 100

Where,

E= epidermal cells

S= number of stomata in the field

Stomatal Frequency/ mm^2 = XY^2 X 10^6/ S

Where,

X = number of stomata in the field

Y = magnification

S = square area of the field

Vein- islet number was determined according to Levin (1929) and veinlet termination number according to Hall and Melville (1954).

Results and Discussion

In the present investigation micro- features namely epidermis, stomata, trichomes, major and minor venation of the selected taxa belonging to the family Apocynaceae have been investigated.

Carissa carandas Linn.

Macro-characters

Leaves opposite, simple, shortly petioles up to 0.3 cm long, glabrous, lamina elliptic or obovate, obtuse or retuse (often shortly- mucronate) mucronulate or muticous, base rounded or subcordate or rarely cuneate, 2.5- 9.3cm X 2.0- 5.5 cm, entire, thinly coriaceous, upper surface shining

dark green, glabrous, midrib impressed and laterals & tertiaries raised when dry; lower surface green , midrib prominently raised; main lateral nerves 8- 26, looping; nervation conspicuous (Table 1).

Micro- characters

Epidermis : Cells of upper epidermal surface tetra to polygonal slightly sinuous walled. Cells of lower surface penta to polygonal straight walled (Fig.1(b)A).

Stomata

Leaves hypostomatous : Stomata predominantly paracytic type; amphiparacytic stomata also observed (Fig.1(b)B,C,D).

Stomatal frequency : 21.5 (Table 2)

Stomatal index : 12.66(Table2)

Trichomes : Not observed on any surface

Venation pattern

Major venation : Pinnate brochidodromous (Fig.1(a))

Minor venation : Vein- islets with & without endings; vein- islets triangular, quadrangular or irregular in shape. Vein-endings simple and of linear type (Fig.1(b)E)

Vein- islets : 8.20 per mm^2

Vein- endings : 15.0 per mm^2

***Alstonia scholaris* R. Br.**

Macro-characters

Leaves verticillate, 3-8 at a node, simple, petiolate, petioles stout; 0.5- 1.2 cm long, glabrous; lamina obovate, elliptic oblong, oblong- lanceolate or oblanceolate, obtuse or retuse; base cuneate and faintly decurrent; 6.0- 20.0 cm X 1.1- 6.5 cm, entire, coriaceous; upper surface dark green, glabrous, lower surface rather pale and curved with a whitish bloom, glabrous, midrib prominantely raised, main lateral nerves 56- 60, closed, nearly horizontal and parallel, joining an intra marginal one; nervation inconspicuous (Table 1).

Table 1. Major venation characterstics in the Apocynaceae

S. No.	Name of species	Number of 2^0 veins along one side of midrib	Range of angles between 1^0 and 2^0 veins	Primary vein	Nature of the midrib on adaxial side	Size of midrib
1.	*Carissa carandus*	7-9	25^0- 45^0	Straight	elevated	Massive
2.	*Alstonia scholaris*	Numerous	5^0- 20^0	Straight	elevated	Stout

Table 2. Micro- anatomical characteristics of the Apocynaceae

Name of the species	Type	Stomata				Trichomes
		Stomatal frequency		Stomatal index		
		Lower	Upper	Lower	Upper	
Carissa Carandus	P, AP	21.5	-	12.66	-	Absent
Alstonia Scholaris	A	9.3	-	9.50	-	Absent

A= Anomocytic, Ap= Amphiparacytic, P= Paracytic

Micro- characters

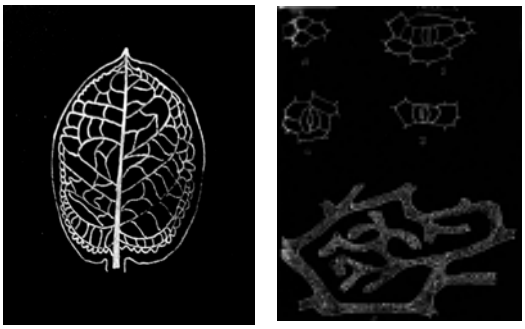
Epidermis : Cells on upper as well as lower epidermal surface tri – to polygonal, straight walled (Fig.2(b)A).

Stomata : Leaves hypostomatous; stomata are pridominently anomocytic type {Fig.2(b)B,C}; other type {Fig.2(b)D}.

Stomatal frequency : 9.50(Table 2)

Stomatal index : 9.3(Table 2)

Trichomes : absent



1(a) 1(b)

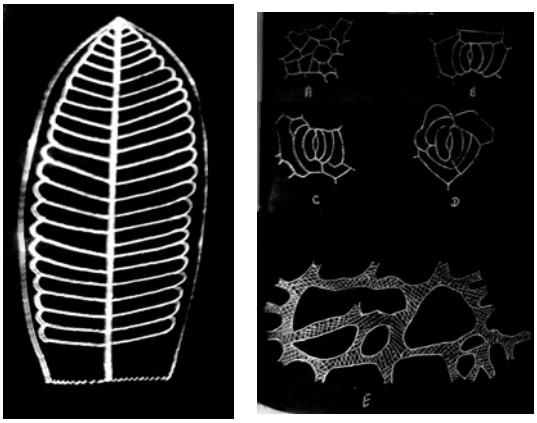
Fig.1.(a) Showing major venation of leaf of *Carissa carandas* L.

1.(b) A- A portion of epidermis
B- Amphiparacytic stomata from lower epidermis
C- Paracytic stomata from lower epidermis
D- Paracytic stomata with one subsidiary transversely divided.
E- A portion of minor venation

Venation pattern

Major venation	:	Pinnate brochidodromous; secondaries run parallel (Fig.2a)
Minor venation	:	Vein- islets imperfect with a large number of linear branched veinlets (Fig.2b-E).
Vein- islets	:	5.90 per mm^2
Vein- endings	:	17.25 per mm^2

In the study it was observed that upper and lower epidermal cells of both the studied taxa are mostly tri- to polygonal. Stomata of different types are noticed in the leaves of the studied taxa. The presence or absence of stomata on the epidermis of the leaves is extremely useful in delineating taxa both at the species and generic level(Gonzalez *et al*, 2004;Kadiri *et al*, 2005/2006). Leaves of both the taxa studied are hypostomatic. A Systematic study of the development and morphology of the stomatal types provides valuable clues regarding the various evolutionary trends among the angiosperm families. The structural features of the individual stomata may be important but equally valuable are their pattern of distribution. A detailed study of microfoliar features revealed that the morphological variations shown among the species of a genus and the different genera of the family are interconnected with the anatomical features too. Wilkins (1971) pointed out that the stomatal index is a useful taxonomic character when comparable leaf areas are used. The taxa collected from different localities of Jaipur showed more or less constant stomatal index that can be used in distinguishing different taxa. According to Stace (1966,1980,1984) the distribution and frequency of stomata are useful in solving problems of systematics. Salisbury (1927) emphasized that the frequency of stomata is high when the size of the epidermal cells is low and that the frequency is low when the epidermal cells are large .



2(a) 2(b)

Fig.2.(a) Showing major venation of leaf of *Alstonia scholaris* R.Br.

Fig.2.(b) A- A portion of epidermis
B, C- Anomocytic stomata from lower epidermis
D- Paracytic stomata from lower epidermis
E- A portion of minor venation

The present study reveals that stomatal index as well as stomatal frequency is high in *Carissa carandas* as compared to *Alstonia scholaris* on the abaxial surface among studied taxa. The present work also shows that in both the taxa leaves are hypostomatous, trichomes are absent and that vein- islets number/ mm^2 & vein- ending termination number/ mm^2 are more or less constant for a species. Certain micromorphological features of leaf epidermis can also be considered as distinct markers for distinguishing slow growing species from fast growing ones. Thus,higher stomatal frequency and stomatal index were characteristics of fast growing species i.e.*Carissa carandas* as compared to *Alstonia scholaris* that is slow growing.

It can be concluded that careful morphological studies of leaf form (such as the venation and epidermal characters emphasized in this paper) will provide better understanding of the relationships of living angiosperms and their taxonomic hierarchy.

References

Borril,M. (1961) Epidermal characteristics in the diploid subspecies of *Dactylis glomerata* L. *Bot J Linn Soc* **56**:453-458.

Chandra,V., Kapoor,S.L.,Sharma,P.L, Kapoor,L.D. (1969) Epidermal and Venation studies in Apocyanaceae I *Bull Bot Surv India* **11**:286-289.

Chandra, V., Mitra , R., Kapoor, S.L., Kapoor, L.D. (1972) Epidermal and venation studies in Apocyanaceae.IV. *Bull Bot Surv India* **14**:76-82.

De Bary, A. (1884) Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Claredendron Press, Oxford,U.K.

Endress, M.E., Bruyans, P.V. (2000) A revised classification of Apocynaceae. 1. *The Botanical Review* **66**: 1-56.

Gonzalez, C. C., M. A. Gandolfo, R. N. Cúneo.(2004) Leaf architecture and epidermal characters of the Argentinean species of Proteaceae. *Int J Plant Sci* **165**: 521-536.

Hagerup, O. (1953) The Morphology and Systematics of the leaves of Eriales. *Phytomorphol* **3**:459-464.

Hall, J. P., Melvelle, C. (1954) Veinlet termination number: Some further observations. *J Pharm Pharmacol* **6:** 129-133.

Hendrian, Middleton, D.J. (1999) A revision of *Rauvolfia* L. in Malesia Blumea **44**: 449-470.

Kadiri, A. B. ,. Olowokudejo ,J.D., O. T. Ogundipe ,O. T. (2005/2006) Some Aspects of Foliar Epidermal Morphology of *Cylicodiscus gabunensis* (Taub.) Harms(Mimosaceae). *Journal Sci Res Dev* **10**: 33 38

Kannabiran, B., Krishnamurthy,K.H. (1972) Morphology of foliar epidermis and taxonomy of genus *Crotolaria. Phytomorphol* **24**:61-68.

Kapoor, S.L, Sharma, P.C., Chandra, V., Kapoor, L.D. (1969) Epidermal and venation studies in Apocyanaceae.II. *Bull Bot Surv India* **11**:372-376.

Karatela, Y.Y., Gill, L.S. (1986) Epidermal morphology and histogenesis in some cover crops in Nigeria. *Afri J Agric Sci* **13**: 103-110.

Leeuwenberg, A.J.M. (1985) Series of revisions of Apocynaceae XV Thou. Agricultural University, Wageningen Papers 85-3: 5-80 .

Leeuwenberg, A.J.M. (1991) Revision of *Tabernaemontana* 1.Royal Botanical Gardens Kew.

Leeuwenberg, A.J.M. (1998) Series of revisions of Apocynaceae XLVII. The genus *Cerbera* L. Agricultural University Wageningen Papers 98-3: 1-64.

Levin, F. A. (1929) The taxonomic value of veinislet areas based upon a study of the genera *Barasma, Cassia, Erythroxylon* and *Digitalis. J Pharm Pharmacol* **2**:17 —43.

Metcalfe, C.R., Chalk L. (1979) Anatomy of Dicotyledons, **1, 2**, Clarendon Press, Oxford, U.K.

Metcalfe, C.R., Chalk L. (1950) Anatomy of Dicotyledons, Vol.III. Clarendon Press, Oxford, U.K.

Metcalfe, C.R., Chalk L. (1979) Anatomy of Dicotyledons, **1,2,** Clarendon Press, Oxford, UK.

Middleton, D.J.(1993) A taxonomic revision of *Willughbeia* Roxb. (Apocynaceae) Blumea **38**:1-24.

Middleton, D.J. (1995a) A taxonomic revision of *Ichnocarpus* (Apocynaceae) Blumea **39**: 73-94.

Middleton, D.J. (1995b) *Baharuia,* a new genus of Apocynaceae from Malesia. Blumea **40**: 443-447.

Middleton, D.J. (1996a) A revision of *Aganonerion* Roxb. (Apocynaceae) Blumea **41**: 37-68.

Middleton, D.J. (1996b) A revision of *Aganonerion* Pierre ex Spire. *Parameria* Benth. and *Urceola* Roxb. (Apocynaceae) Blumea **41**: 69-122.

Middleton, D.J.(1996c) A revision of *Aganosma* (BI) G. Don (Apocynaceae). *Kew Bulletin* **51**: 455-482.

Middleton, D.J. (1997) A revision *Parsonsia* R. Br. in Malesia. Blumea **42**: 191-248.

Middleton, D.J. (1999) Apocynaceae in Flora of Thailand **7**(1): 1-152.

Middleton, D.J. (2000) Revision of *Alyxia* (Apocynaceae). Part I: Asia and Malesia. Blumea **45**: 1-146.

Mukherjee, K.K., Roy, M., Saha P.K., Ganguly, S. N. (2000) Surface Morphology of Tea (*Camellia sinensis* L.) leaves. *Phytomorphol* **50**: 125-131.

Nyawuame, H.G.H., Gill L.S. (1991) Stomatal morphology and effects of potassium chloride, sodium chloride and abscisic acid in the stomatal regulation of two species of *Commelina* L. *Pak J Weed Sci Res* **4**: 48-58.

Nyawuame, H.G.H., Gill L.S.(1990) Epidermal morphology and histogenesis in some crop plants. *Nig J Agron* **4**: 6-10.

Ogundipe, O.T., Akinrinlade, O. (1999) Epidermal micromorphology of some species of *Albizia* Durazz (Mimosaceae). *Phytomorphol* **48**: 325-333.

Praveen, N.S., Murthy K.S.R., Pullaiah, T. (2000) Leaf epidermal characters in *Crotalaria* sp. (Papilionoideae) from Eastern Ghats. *Phytomorphol* **50**: 205-212.

Ramayya, N., Vanaja,V.(1979) Development of a Triple acid treatment method for separation of firmly adherent foliar dermides. *Geobios* **6**:5-8.

Rudjiman. (1986) A revision of *Beaumontia* Wallich, *Kibatalia* G. Don and *Vallariopsis* Woodson. Agriculture University Wageningen papers 86-5: 36.

Salisbury, E. J.(1927) On the causes and ecological significance of stomatal frequency with special reference to the wood land flora. *Phill Trans Roy Soc London* **216**:1-65.

Salisbury, E. J.(1932) The interpretation of soil climate and the use of stomatal frequency as an interesting index of water relation to the plant. *Beih Bot Zentralb* **49**:408-420.

Sharma, P.C., Chandra, V., Kapoor, S.L. and Kapoor, L.D. (1970) Epidermal and venation studies in Apocyanaceae.III. *Bull Bot Surv India* **14**:92-96

Sidiyasa, K. (1998) Taxonomy, Phylogeny and wood anatomy of *Alstonia* (Apocynaceae). *Blumea suppl* **11**:1-230.

*Stace, C. A. (*1966) *The use of epidermal characteristics in Phylogenetic considerations. New Phytologist* **65***:* 304–*318.*

Stace, C.A. (1980) Plant Taxonomy and Biosystematics. Edward Arnold (Australia) Ptv. Ltd., Melbourne, Australia.

Stace, C.A. (1984) The taxonomic importance of the leaf surface. In: Heywood, V.H., Moore,D.F. (Eds.) Current concept of Plant Taxonomy, Academic Press, London. pp.31-39.

Tomblinson, P.B. (1959) Anatomical approach to the classification of the Musaceae. *Bot J Linn Soc* **5**:779-809

Wilkins, M . B. (1971) Physiology of Plant Growth and Development. McGraw-Hill, New York. pp. 54-78.