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Foliar stomatal characters as supplementary tool for identification of rattans: A case study in selected *Calamus* species of the Western Ghats, Kerala

K. C. Kariyappa, Joemon Jacob and **N. Mohanan***
Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, Kerala, India

Abstract: Eight species of *Calamus* from the Western Ghats of Kerala were studied for their epidermal characters. The study reveals that, *Calamus* species show marked differences in the epidermal features in one or more characters, depending on the habitat and leaf type. These characters along with other morphological features could be used as a tool for identification of the species even in vegetative stage.

Keywords: Epidermal cell, stomata, stomatal frequency, stomatal index.

INTRODUCTION

The significance of epidermal features in traditional taxonomy has long been recognized, because the variations within the species, genus or family are usually reflected in anatomical features as well. Systematic studies on the development and morphology of stomata and associated epidermal cells are proving to be increasingly important from the taxonomic point of view, as they may be expected to give clue to various evolutionary trends and help to assign taxa of uncertain affinity to their true position. The studies on stomatal features of monocotyledons were initiated by various scholars like Stebbins and Jain (1960), Stebbins and Kush (1961), Tomlinson (1960, 1961, 1974), Ghose and Davis (1973, 1974). However, in Arecaceae, particularly in rattans, such works are comparatively less except those of Tomlinson (1961), Mahabale and Nandini (1963), Ghose and Davis (1974), Basu and Basu (1978), Henderson (2006).

Calamus (Arecacea), a genus of tropical and subtropical distribution, comprises about 374 species all over the world (Govaert and Dransfield, 2005). Of these, 17 species are distributed throughout the Western Ghats and coastal regions of Kerala (Renuka, 2000; Renuka and Sreekumar, 2006; Rangan *et al.*, 2003). In the present paper foliar

* To whom correspondence should be addressed; E mail: nmohanan59@rediffmail.com

stomatal types, epidermal cells and their distribution in eight species of rattans, *Calamus gamblei, C. brandisii, C. delessertianus, C. thwaitesii, C. hookerianus, C. dransfieldii, C. rivalis* and *C. travancoricus* are presented.

MATERIALS AND METHODS

Fresh materials from identified natural populations of each species were used for the study. Middle leaflets of the third leaf from the tip of the plant and middle part of the leaflets were used to prepare epidermal peelings uniformly in all the species. Epidermal peeling was taken by scrapping the leaf portion with razor on a microslide after treating with 10 per cent sodium hypochlorite solution for two minutes. The peelings thus obtained were dipped in water for 5 min and then transferred to a supersaturate solution of chloral hydrate till rendered transparent. After thorough washing in water, these peelings were stained in aqueous safranin solution and mounted in 5 per cent glycerine.

For each species, two peels of both surfaces were taken and ten microscopic fields of each surface of each specimen were studied. The data were based on average of 20 readings on each surface of each specimen. The quantitative data were obtained by Nikon ECLIPSE 80i Microscope and photos were taken by Nikon Digital DXM 1200 F camera attached to the Microscope. The description of the stomatal type in the study is based on Stebbins and Kush (1961). The stomatal frequency and epidermal frequency were calculated in mm^2.

RESULTS

The data on quantitative foliar features are given in the Tables 1, 2, and 3. It is found that the epidermal cells of all the species studied are similar, except in *C. thwaitesii.* The cells are rectangular in shape and are arranged in linear pattern, whereas in *C. thwaitesii* the upper and lower epidermal cells have no definite shape and pattern of arrangement (Figs.1, 2). The cell wall in all the species is cutinized and has dentate anticlinal walls with straight and sinuous end walls. The upper epidermis of *C.*

Table 1. Qualitative foliar features of *Calamus* species

Species	Epidermal cell shape		Epidermal cell wall		Stomatal complex	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
C. brandisii	R	Rectangle	D	D	P	P
C. gamblei	R	Rectangle	D	D	P	P
C. thwaitesii	I	irregular	S	I	P	P
C. hookerianus	R	Rectangle	D	D	P	P
C. rivalis	R	Rectangle	D	D	P	P
C. delessertianus	R	Rectangle	D	D	P	P
C. travancoricus	R	Rectangle	D	D	P	P
C. dransfieldii	R	Rectangle	D	D	P	P

* R: rectangle; I: irregular; D: dentate; S: sinuous; P: paracytic.

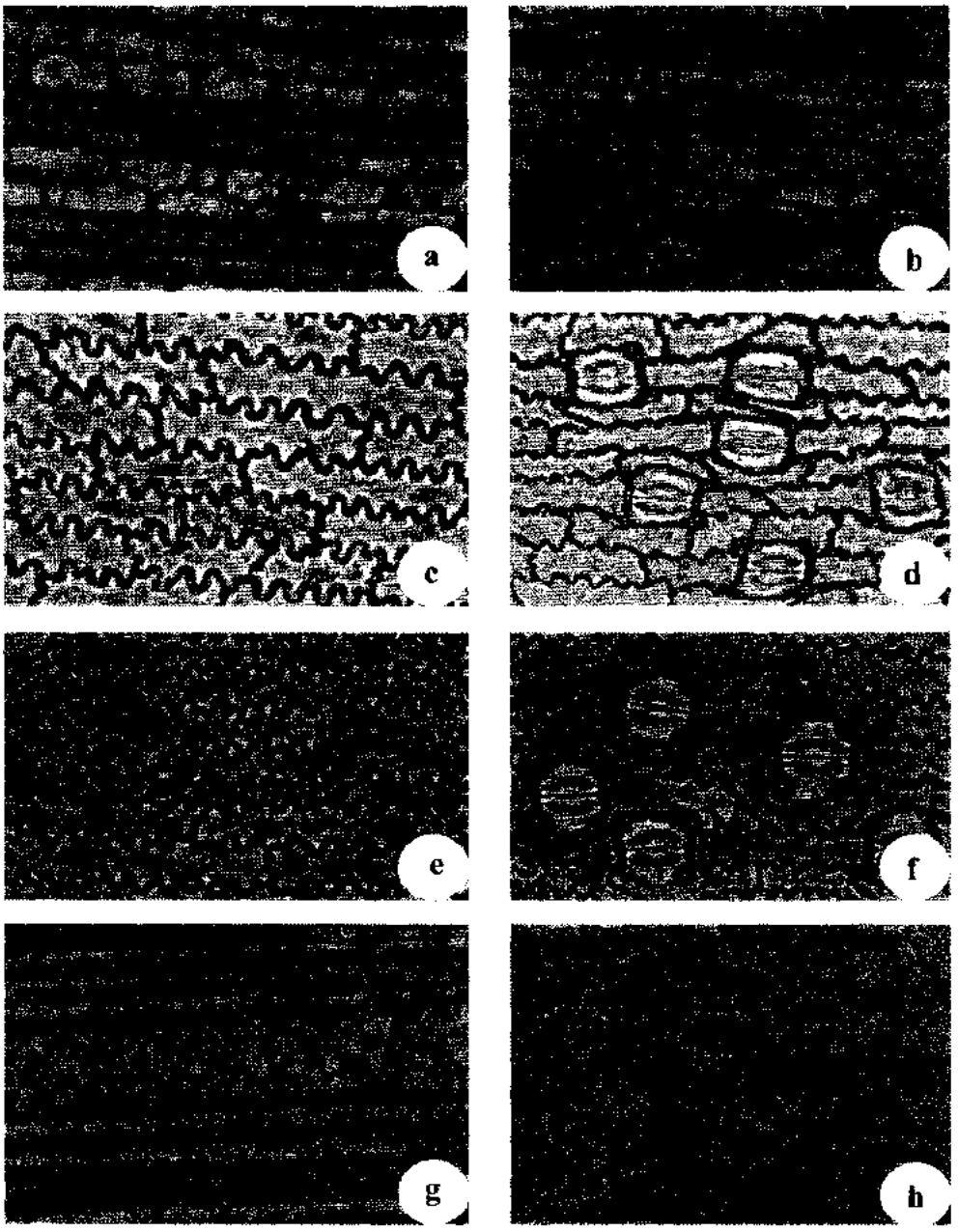

Plate 1

Figure 1. a, b: *C. brandisii*; c, d: *C. delessertianus*; e, f : *C. dransfieldii*; g, h: *C. gamblei* (a, c, e, g: adaxial epidermis; b, d, f, h: abaxial epidermis)

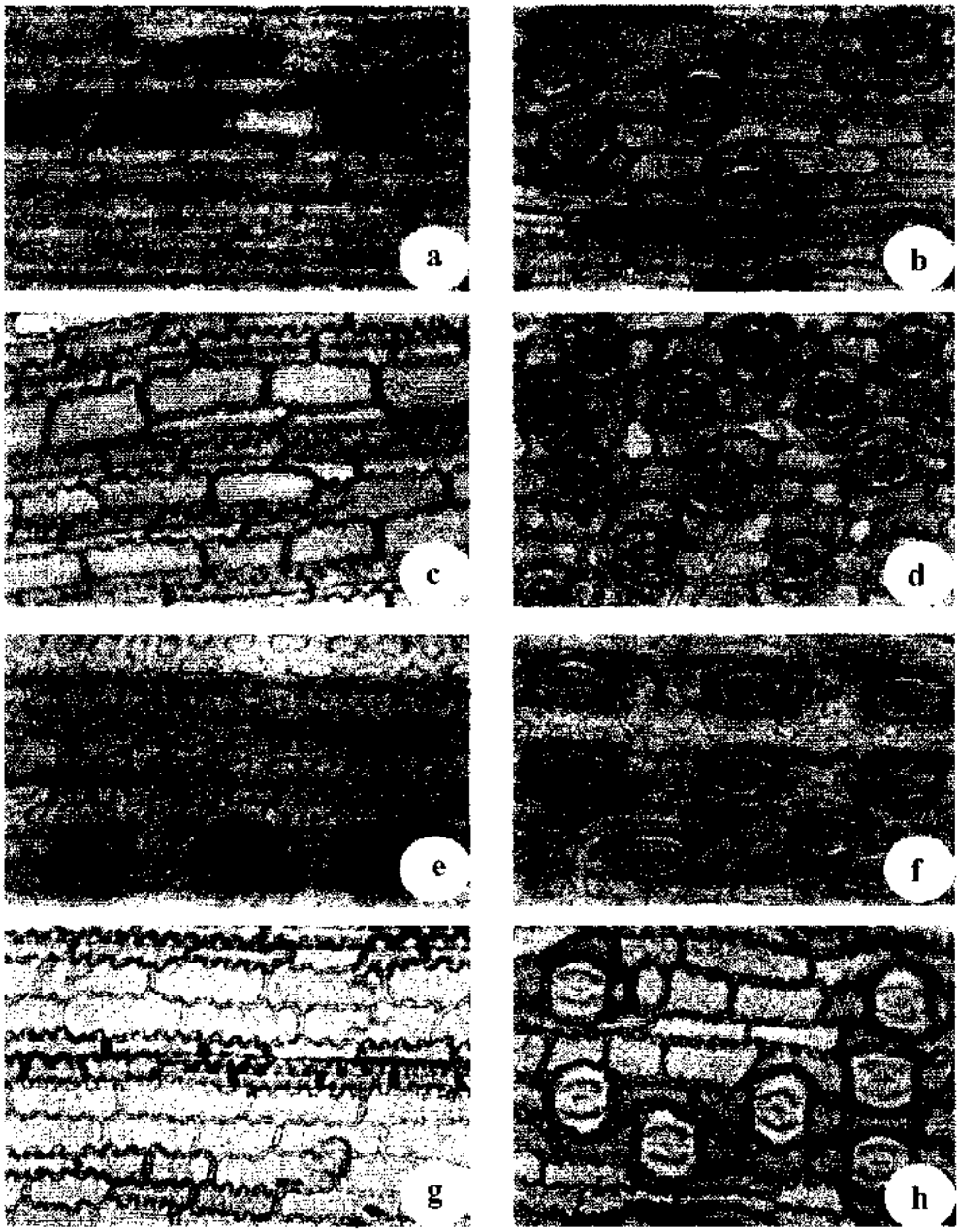

Plate 2

Figure 2. a, b: *C. hookerianus*; c, d: *C. rivalis*; e, f: *C. thwaitesii*; g, h: *C. travancoricus* (a, c, e, g: adaxial epidermis; b, d, f, h: abaxial epidermis)

Table 2. Quantitative foliar features

Species	Surface	Epidermal cell frequency / mm²	Stomatal frequency / mm²	Stomatal index
C. brandisii	U	2157.45	14.27	0.56
	L	1811.4	413.25	18.56
C. gamblei	U	1955.67	5.1	0.025
	L	1258.60	237.96	15.94
C. thwaitesii	U	3475.67	17.32	0.58
	L	—	631.85	—
C. hookerianus	U	2507.00	1.53	0.16
	L	1912.35	385.22	16.76
C. rivalis	U	2149.82	21.91	0.99
	L	1912.36	929.33	36.26
C. delessertianus	U	2155.41	3.06	0.14
	L	1742.68	254.78	12.76
C. travancoricus	U	2547.11	15.29	0.60
	L	2445.86	402.55	14.29
C. dransfieldii	U	1926.11	5.1	0.26
	L	1676.43	229.30	12.03

* U: upper surfare; L: lower surface; —: not calculated

thwaitesii is highly cutinized, the cells are with reduced lumen and this layer has a sclerenchymatous appearance. The lower epidermal cells are similar to the upper epidermal cells in most species except in *C. thwaitesii,* where the lower epidermal cells are irregularly cutinized and difficult to distinguish the cells. The size of the epidermal cells also shows some variations (Table 3). The maximum mean length is observed in *C. delessertianus* (54.5 μm) and lowest in *C. thwaitesii* (17.3 μm). The highest mean width is observed in *C. brandisii* (17.5 μm) and lowest in *C. hookerianus* (8.5 μm).

The upper and lower epidermal cell frequency ranges from 1926.11 to 3475.67/mm² and from 1258.60 to 2445.86/mm² respectively (Table 2). *C. thwaitesii* has the highest adaxial value (3475.67/mm²) followed by *C. travancoricus* (2547.11/mm²). The least adaxial epidermal cell frequency is observed in *C. dransfieldii.* The abaxial epidermal frequency is high in *C. travancoricus* (2445.86/mm²) followed by *C. rivalis* (1912.36/mm²). The lowest value is in *C. gamblei.*

All the species studied are amphistomatic in nature. Stomata are more on the lower epidermis and the upper epidermis consists of only a few rows of stomata on the margin of the lamina. The lower epidermis consists of stomata in linear files on the intercostal regions. The stomata are monocot II (paracytic) type with two lateral subsidiary cells parallel to the guard cells. The terminal cells vary in length from short to long and over arch the guard cells in all the species. The terminal cell is very short in *C. thwaitesii*. Stomatal frequency varies in different species on both upper

Table 3. Quantitative foliar characters

Species	Epidermal cell size (µm) Adaxial		Epidermal cell size (µm) Abaxial		Stomatal complex size (µm)	
	Length Min. (Mean ± STD Error) Max.	Breadth Min. (Mean ± STD Error) Max.	Length Min. (Mean ± STD Error) Max.	Breadth Min. (Mean ± STD Error) Max.	Length Min.(Mean ± STD Error) Max.	Breadth Min. (Mean ± STD Error) Max.
C. brandisii	20 (35±10.60) 45	10 (17.5±3.29) 15	20(35±10.60) 45	10(17.5±3.29) 15	20(21.5±2.28) 22.5	17.5(19±1.44) 20
C. gamblei	25(35±10.87) 75	10(12.95±2.20) 15	25(35±10.87) 75	10(12.95±2.20) 15	22.5(25.75±2.04) 27.5	15(15.75±1.44) 17.5
C. thwaitesii	10(17.3±6.75) 27.5	7.5(12±1.1) 15	X	X	15(23.3±5.10) 27.5	12.5(13.8±1.18) 15
C. hookerianus	17.5(34.8±8.97) 55	10(13.3±2.20) 17.5	25(47.75±17.85) 72.5	5(8.5±3.20) 7.5	22.5(24.8±0.00) 25	15(19.5±3.20) 22.5
C. rivalis	15(33.5±13.55) 45	10(12.3v1.28) 15	10(22.4±9.49) 40	9(10.1±1.00) 12	17(20±1.24) 21	10(11.9±1.25) 14
C. delessertianus	25(54.5±10.18) 75	10(14.2±2.92) 20	15(44.7±18.59) 75	10(12.1±1.43) 15	22(23.7±0.48) 25	8(9.6±0.96) 11
C. travancoricus	22(35.2±6.97) 50	9(12.3±2.86) 15	15(30.5±18.39) 55	10(12.8±2.59) 16	20(20±0.00) 20	20(20±0.00) 20
C. dransfieldii	20(43.5±16.49) 80	10(14.6±1.48) 17	25(46±15.09) 90	12(12.9±1.48) 15	25(25±0.00) 25	20(20±0.00) 20

Min- minimum; Max- maximum; X.- not measured

and lower epidermis (Table 2). The adaxial stomatal frequency is maximum in *C. rivalis* (21.92/mm^2) followed by *C. thwaitesii* (17.32/mm^2) and least in *C. hookerianus* (1.53/mm^2). The abaxial stomatal frequency is highest in *C. rivalis* (929.33/mm^2) followed by *C. thwaitesii* (631.85/mm^2) and least is in *C. dransfieldii* (229.30/mm^2). The stomatal index of adaxial surface is maximum for *C. rivalis* (0.99) followed by *C. thwaitesii* (0.58) and least in *C. gamblei* (0.025) (Table 2). The abaxial value is maximum for *C. rivalis* (36.26) followed by *C. brandisii* (18.56) and least in *C. dransfieldii* (12.03). Stomatal index on both surfaces showed variations (Table 2). The upper stomatal index is maximum in *C. rivalis* (0.99) followed by *C. thwaitesii* (0.55) and minimum in *C. gamblei* (0.025). The abaxial stomatal index is high in *C. rivalis* (36.26) followed by *C. brandisii* (18.56) and minimum in *C. dransfieldii* (12.03).

The size of the stomata on both surfaces are similar in nature and showed some variation among the species (Table 3). The highest length is noticed in *C. gamblei* (27.5 μm) and lowest in *C. thwaitesii* (15 μm). The average length ranges from 20 μm to 25.75 μm. Width is maximum in *C. hookerianus* (22.5 μm) and minimum in *C. delessertianus* (8 μm). The average width ranges from 9.6 μm to 20 μm.

DISCUSSION

Stomatal distribution patterns are considered to have taxonomic value (Stebbins and Kush, 1961). The result obtained from this study reveals variation in their stomatal distribution among the rattan species studied. The stomatal type in all the species is monocotyledon II (paracytic type) as reported by Stebbins and Kush (1961). The distribution of stomata is as observed by Tomlinson (1961), on both upper and lower surfaces, and in the upper surface it is restricted to the margin of the blade. Distribution, size and frequency of stomata have been reported to be specific for a genus or a species (Miller, 1938) and hence these characters are considered as potential tools in taxonomy and phylogeny (De Bary, 1884; Stace, 1965; Rajagopal, 1979). *C. rivalis* shows maximum stomatal frequency as it is a plant in marshy habitat. However, stomatal frequency of *C. thwaitesii* is also high (Table 2). This species is seen in comparatively dry habitat and open forest. Here the increase in stomatal density is a morphological adaptation to xeric environment (Esau, 1965; Fahn, 1974; Devlin and Witham, 1983; Noggle and Fritz, 1983). The xeric nature is confirmed by the presence of thick deposition of cuticle, especially on the upper epidermis (Tomlinson, 1961). Plants grown in high light intensity have amphistomatous leaves with slightly higher stomatal density on the abaxial surface, than plants from low light intensity (Cutter, 1978). The lower epidermal stomatal frequency of *C. delessertianus, C. gamblei* and *C. dransfieldii* are similar as they grow in similar climatic conditions. The stomata on the upper epidermis in *Calamus* species. are restricted to near the large vein and the margin of the lamina. So, the comparison of stomata on the upper surface is of not much significance.

The epidermal cell walls are sinuous in nature due to the deposition of cuticle in all the species, except in *C. thwaitesii*, where it is smooth on the adaxial surface and irregular on the abaxial surface. Difference in cuticular patterns is taxonomically useful even at the generic or species level (Fahn, 1974). The difference in cuticular thickening in *C. thwaitesii* helps to distinguish it from other rattan species. The epidermal cell shape is similar in all the species studied except *C. thwaitesii*, where the upper epidermal cells are smaller in size, the lower ones are irregularly cutinized and compressed, to making difficult to distinguish each cell. Hence, the lower epidermal cell frequency and index could not be recorded. The anticlinal walls are dentate as noticed by Henderson (2006) in some *Calamus* species. The end wall of *C. gamblei* and *C. dransfieldii* are sinuous, where the other species have straight wall which differs from the observation of Henderson (2006).

Another interesting feature revealed in this study is the presence of a correlation between the stomatal frequency and the type of leaf and leaflets arrangement. The adaxial and abaxial stomatal frequency of *C. brandisii* and *C. travancoricus* with cluster arrangement of leaflets is almost same. Similar situation is observed in *C. gamblei*, *C. delessertianus* and *C. dransfieldii*, where the leaflets are pinnately arranged. The epidermal cell frequency and stomatal frequency are slightly higher in *C. hookerianus*. This may be due to the small size of the epidermal cells (Table 3) and open habitat than the other three species. This helps to distinguish it from other plants with pinnately compound leaf.

The size of the stomata in each species of monocotyledon is much more consistent than in the dicotyledons and may be used as a relatively reliable character (David *et al.*, 1965). The data from the present study also show such variations, which can be used for distinguishing the species. The smallest size is observed in *C. rivalis* and more or less isodiametric stomatal complex is seen in *C. travancoricus*. The biggest size is recorded in *C. dransfieldii* (Table 3). This helps to distinguish it from *C. gamblei* and *C. delessertianus*.

On the basis of the present study an artificial key developed is given below:

1a Plants with cluster arrangements of leaves 2
1b Plants with regular arrangement of leaves.....3
2a Leaf with highly cutinized adaxial epidermal cells and
with abaxial stomatal frequency more than 600..... *C. thwaitesii*
2b Leaves with adaxial stomatal frequency below 500.....4
3a Leaves with abaxial stomatal frequency more than 300 .5
3b Leaves with abaxial stomatal frequency below 300..... 6
4a Leaves with abaxial epidermal frequency below 2000 ...*C. brandisii*
4b Leaves with abaxial epidermal frequency above 2000
with isodiametric stomata.....*C. travancoricus*

5a Leaves with abaxial stomatal index below 20 ..... *C. hookerianus*
5b Leaves with abaxial stomatal index above 20 .....*C. rivalis*
6a Leaves with adaxial epidermal frequency above 2000 ....*C. delessertianus*
6b Leaves with adaxial epidermal frequency below 2000 7
7a Leaves with abaxial epidermal frequency above 1500.....*C. dransfieldii*
7b Leaves with abaxial epidermal frequency below 1500.....*C. gamblei*

Epidermal characters along with other morphological characters are useful in identification of the species (Patil and Patil, 1987). The present study reveals such variation within the species in one or more characters that can be used for the identification. In the vegetative phase, identification of rattans is very difficult, some times even for a taxonomist (Renuka, 2000). It will be made easier, if the morphological characters along with the epidermal characters are considered. Hence, more studies are needed in this area for the easy identification of rattans employing taxonomic key based on epidermal characters and morphological characters.

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