

Comparative macroscopic and microscopic foliar characterization of twelve species belong to four genera in family Costaceae

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Received: September 09, 2018

Accepted: October 22, 2018

ABSTRACT

The spiral Ginger family Costaceae is composed of 7 genera and above 100 species and is mostly confined to tropical regions of Asia, Africa, Central America and South America. Most of the taxa have ornamental potential with spirally twisted stem, attractive spikes, and often with colorful foliage. Many of them are widely cultivated in gardens. 12 species belong to 4 genera, viz, *Costus*, *Hellenia*, *Monocostus* and *Tapinochilos* were selected for the study leaves of this species were morphologically characterised. The leaves are sessile to shortly petiolated with a closed tubular sheath; lamina entire, lanceolate to linear, generally with a pointed apex. 11 qualitative and 5 quantitative macroscopic characters were accessed and compared. Characters of stomata and trichomes were determined by microscopic observation. Though the stomata are parasitic in all the taxa studied, the stomatal index varies from 5.2-9.96%. The type, distribution and density of trichomes were vary considerably between the species. The systematic significance of foliar characters is also discussed.

Keywords: Micromorphology, *Costus*, Stomatal index, Trichomes

1. INTRODUCTION

Costaceae belongs to the order Zingiberales is commonly known as 'Spiral Ginger Family has a pantropical distribution. It contains many horticulturally as well as economically important plants.

Through floral characters were mainly used to for taxonomic identity, there were several instances, where foliar characters, both macroscopic and microscopic have successfully utilized to solve taxonomic problems and also for phylogenetic relationships among different taxon. (Sibichen, 2011).

The importance of micromorphological features for taxonomic consideration in Angiosperms was well established by several authors such as Ramayya, (1972), Tomilson (1979), Ogundipe and Akinrinlade (1998), Praveen et al (2000) etc . As floral variations, foliar variations among the species are also important tool to identify a Solereder, 1908; Metcalfe and Chalk, 1950; Stace, 1965; Paliwal, 1969; Ahmed, 1979; Rajagopal, 1979). The presence of peculiar type of trichomes can frequently delimit species, genera or even whole families (Metcalf et al., 1950). In the present investigation, twelve species belonging to four genera of Costaceae, available in Southern India were subjected to characterize with respect to their foliar characters macroscopic and microscopic, leading to the identity of the species.

2. MATERIALS AND METHODS

Characterization of taxa: The fresh leaves of selected twelve species were collected from the Ginger garden of JNTBGRI Palode. The leaves were collected randomly from each species, during February 2018. The species included in the study are

Costuserythrophyllus Loes., *C. quanaiensis* Rusby ., *C. malortianus* H.Wendl., *C. pictus* D. Don., *C. spicatus* (Jacq.) Sw., *C. spiralis* (Jacq.) Sw., *C. stenophyllus* Standl. And L. O.Williams., *C. varzeorum* Mass, *C. woodsonii* Mass, *Hellenia speciosa* (J. Koenig ex Smith) S.Dutta, *Monocostus uniflorus* (Poepp. ex Petersen) Mass, *Tapinochilos anannasae* (Hassk.) K.Schum

Data on 10 fresh leaves at the central portion of the shoot of each species were observed and mean value of the quantitative characters were documented

Quantitative parameters such as size, thickness, number of major veins and petiole length were studied, The quantitative were measured with an ordinary scale and digimatic verniercaliper (CD- 6 CS), where as the qualitative characters were observed with the naked eye as well as a hand lense.

The type or nature of stomata and trichomes were observed from the epidermal peeling of abaxial surface of fresh leaves sained with diluted safrannine solution kept for 1-2 minutes. The peelings was gently washed using distilled water, mounted on a clean glass slide under the covreslip and observed through binocular microscope (Leitzwetzler Germany 512 582) under 25x magnification .Stomatal index was calculated by applying the formula (Dichler, 1974).

$$\text{Stomatal Index (SI)} = \frac{\text{Total numer of stomata (S)} \times 100}{\text{total number of epidermal cell (E)} + \text{number of stomata (S)}}$$

3. RESULTS AND DISCUSSION

3.1 Qualitative parameters:

The qualitative characters observed were shape of leaf, leaf tip, leaf base, texture and colour of both upper and lower side of lamina and petiole, colour and nature of leaf sheath.

In the present observation, leaves of *Costusmalortianus*, *C.spicatus* and *Tapeinochilos ananassae* were oblanceolate. Oval- lanceolate, small leaves were observed specifically in the genus *Monocostus*. The leaf shape was elliptic- obovate and narrow linear- lanceolate leaves in *C. varzearum*, *C. stenophyllus*, respectively, which are very characteristic. In all the other six species it was elliptic-lanceolate. Most of the species were with acuminate leaf tip and attenuate leaf bases. The genera *Monocostus* and *Tapeinochilos* were exceptions with round leaf bases. Most lamina are symmetrical in nature whereas slightly oblique nature was observed in four species such as *Costus erythrophyllus*, *C. malortianus*, *C.varzearum* and *Tapeinochilos ananassae*. Colours of upper side of lamina in most of the species were observed as dark green except in *Costus guanaiensis*, where it was dull green. In *Costusmalortianus* the leaves are strikingly pale green often with feeble green bands. Lower side of lamina in almost all species were pale green where as in *Costus erythrophyllus* and *C. varzearum* it was maroon colour. Generally the petiole were green in colour but maroon- green in *C. erythrophyllus* and green with pinkish margin in *C. pictus*. The nature of leaf sheath was another important qualitative character observed as shown in table 1. It was usually closed and fleshy in most of the species, where as in *C. guanaiensis*

Table- 1 Qualitative foliar macroscopic characters

Plant Species	Colour Of Petiole	Texture On Lower Side Of Lamina	Texture On Upper Side Of Lamina	Nature Of Leaf Sheath	Leaf tip	Leaf Base	Colour Of Leaf Sheath
<i>Costus erythrophyllus</i>	Green maroon	Shining	Smooth	Closed and fleshy	Acuminate	Attenuate	Maroon
<i>C. guanaiensis</i>	Green	Smooth	Smooth	Closed, bulged at the tip	Acuminate	Attenuate	Pale green
<i>C. malortianus</i>	Green	Densely pubescent	Densely pubescent	Closed and fleshy	Acuminate	Slightly auriculate	Green
<i>C. pictus</i>	Green with pinkish appear on the margin	Smooth	Smooth	Closed and fleshy	Caudate	Attenuate	Green- pink
<i>C. spicatus</i>	Green	Smooth	Smooth	Closed and fleshy	Acute	Attenuate	Green
<i>C. spiralis</i>	Green	Fuzzy	Smooth	Closed and fleshy	Caudate	Cuneate	Green
<i>C. stenophyllus</i>	Green	Rough	Long pubescent	Tubular, extended and open	Narrowly acuminate	Attenuate	Brown
<i>C. varzearum</i>	Green	Smooth	Smooth	Closed and fleshy	Acuminate	Attenuate	Green colour shaded on Maroon
<i>C. woodsonii</i>	Green	Smooth	Smooth	Closed and fleshy	Acuminate	Slightly auriculate	Green
<i>Helleniaspeciosa</i>	Green	Densely Hairy	Smooth	Closed and fleshy	Acuminate	Cuneate	Purplish green or red
<i>Monocostus uniflorus</i>	Green	Smooth	Glossy	Closed and fleshy	Acuminate	Rounded	Green
<i>Tapeinochilos ananassae</i>	Green	Fuzzy	Smooth	Closed and papery	Acuminate	Rounded	Green- brown

leaf sheaths were bulged towards the tip, and that is the reason for its common name Budha's belly Ginger. In *C. stenophyllus* the leaf sheaths were tubular and extended and open as a ligule with brown colour. In the the genus *Tapeinochilos* thin and papery leaf sheath was observed. Colour and structure of the leaf sheath showed great variations among the species. It was maroon in *C. erythrophyllus*, green with a maroon tinge in *C.varzearum*, brown in *C. stenophyllus*, pale green in *C. guanaiensis* and purplish green or red in *Helleniaspeciosa* and green colour in *T. ananassae* but turning brown when mature. In all other species the sheath were green coloured.

3.2 Quantitative parameters: The lamina of *Costus guanaiensis* were recorded as largest among the species studies ca 36.14 x 13.19 cm, and also observed great variations within the species ranges from 20-50 x 8-16 cm. The genus *Monocostus* characterized with smallest lamina (ca 6.35 cm) and sessile. The narrowest laminas were observed in *C. stenophyllus*. Longest and shortest petioles were recorded in *Costus woodsonii* (ca.1.98 cm) and *C. pictus*(ca.0.4 cm), respectively. The thickness of lamina were observed maximum in

C. malortianus (0.525 mm) and minimum was observed in *C. stenophyllus* (0.25 mm). The number of pair of major veins on the lamina is also varied in different species (Table 2). In *C. stenophyllus* and *Monocostus* the veins are feeble. The number of pairs of veins was found maximum in *C. guanaiensis* and *C. spiralis* (8 pairs each in average).

3.3 Microscopic parameters

a. Stomatal studies: In the present study the microscopical characterization in all species revealed that the type of stomata is paracytic. In paracytic stomata the subsidiary cells of the stomata will be parallel to the guard cells. Obembe (2009) also reported that in the genus *Costus* the stomata are paracytic type. No other reports on stomatal studies were found in this family Costaceae. The average number of stomata and the epidermal cells within a field under 25x binocular microscope is another important parameter studied. It showed a variation ranging from 9/140 in *Monocostus uniflorus* to 85/800 in *Costus woodsonii*. The stomatal index values were also determined as shown in Fig (1). It showed a variation ranging from 5.20 in *C. spiralis* and 9.60 in *C. woodsonii* as recorded in the present study.

The above discussed stomatal parameters have great significance in taxonomy to establish the identity of taxon, because of the specificity within the species with respect to the number of stomata, epidermal cells and stomatal index. In higher plants anatomical characteristics such as type of stomata and stomatal index can be used to establish systematic divisions. Stomatal parameters can also be used to suggest phylogenetic relationships within the plants in general (Metcalfe and Chalk, 1960; Metcalfe, 1969). The role of stomatal index in systematic work to delimit species, because of its constancy for any given species has been reported by (Cutler, 1984; Abdulrahmaan and Oladele, 2003; Aworinde et al., 2009).

b. Trichome studies: In microscopical studies, the characterisation of trichomes was also found to be a significant parameters. The selected species showed much diversity in size and structure of trichomes which provides one of the most important taxonomic characters from foliar studies

Though Tomlison (1962) pointed out that hairs (trichomes) in Costaceae are multicellular, uniseriate and unbranched. In the present investigation two types of trichomes were observed in Costaceae such as unicellular and multicellular. In the selected 12 species, only in 6 species trichomes were observed, where the rest are glabrous. Presence or absence of trichomes were an important parameters to distinguish the identity of a taxon. The distribution and structure of foliar trichomes and petiole expressed considerable variations. Out of this six species possess trichomes, *Costus erythrophyllus*, *C. spiralis* and *Tapeinochilos ananassae* were with unicellular trichomes as shown in (Table; 3) and in *Costus malortianus*, *C. stenophyllus* and in *Hellenia speciosa* the trichomes are multicellular. The present investigation, the longest trichome was observed in *C. stenophyllus* restricted to upper side of lamina only which are multicellular, uniseriate, unbranched and possess over 13 -16 cells. In *C. malortianus* numerous multicellular trichomes were observed on entire lamina and petiole where as in *H. speciosa* trichomes were observed in lower side of lamina and petiole as shown in figure 2. The shortest unicellular trichomes were seen in *Tapeinochilos ananassae* which are restricted to lower side of the lamina. In *Costus erythrophyllus* and *C. spiralis* the unicellular hairs are distantly distributed only in lower side. In *C. spiralis*, the petiole is densely pubescent.

Table 2:- quantitative foliar characters

Name of the Plant Species	Length of Petiole (cm)	Length of Lamina (cm)	Breadth of Lamina (cm)	Thickness of Lamina (mm)	No. of Pairs of Major veins
<i>Costus erythrophyllus</i>	0.96	18.27	8.53	0.519	5
<i>C. guanaiensis</i>	1.28	36.14	13.19	0.36	8
<i>C. malortianus</i>	1.6	22.7	12.44	0.525	6
<i>C. pictus</i>	0.4	27.66	8.85	0.379	5
<i>C. spicatus</i>	0.95	31.09	8.06	0.475	7
<i>C. spiralis</i>	0.78	32.59	11.49	0.361	8
<i>C. stenophyllus</i>	1.06	25.18	2.18	0.25	0
<i>C. varzearum</i>	1.26	30.92	11.21	0.399	7
<i>C. woodsonii</i>	1.98	23.14	9.28	0.477	6
<i>Hellenia speciosa</i>	0.53	23.96	8.84	0.333	6
<i>Monocostus uniflorus</i>	0.0	6.35	3.15	0.424	0
<i>Tapeinochilos ananassae</i>	0.46	18.31	6.33	0.281	3

Fig. 1. Graphical representation on number of stomata and stomatal index in the leaves of 12 species studied

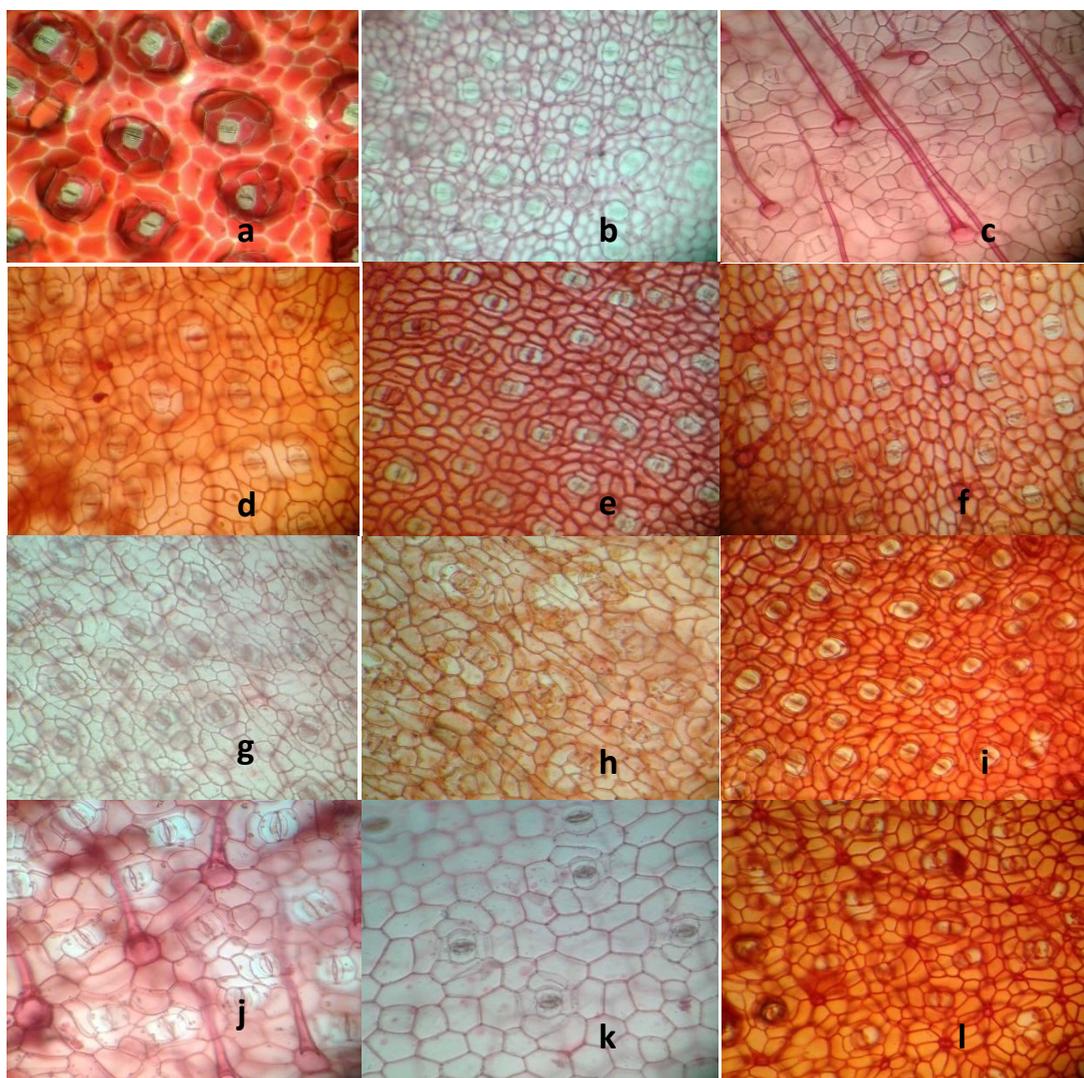
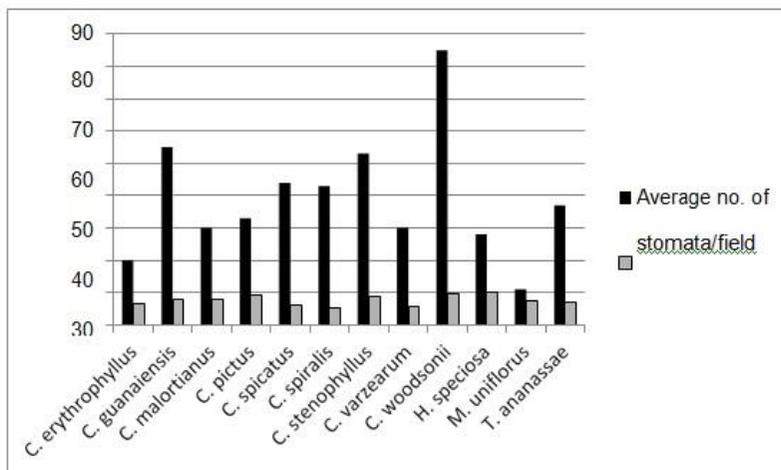


Figure2: Microscopic view (25x) of abaxial epidermis showing stomata of
a. *Costuserythrophyllus*; b. *Costusquanaiensis*; c. *Costusmalortianus*; d. *Costuspictus*;
e. *Costusspicatus*; f. *Costusspiralis* ; g. *Costusstenophyllus* ; h. *Costusvarzearum*; i. *Costuswoodsonii*; j.
***Helleniaspeciosa*; k. *Tapeinichilosananassae*; l. *Monocostusuniflorus*.**

Table 3; Microscopic observation

SL. No	Name of species	Trichomes	Adaxial side of lamina	Abaxial side of lamina	Petiole
1	<i>Costus erythrophyllus</i>	Unicellular, uniseriate and unbranched	Glabrous	Distantly	Glabrous
2	<i>C. guanaiensis</i>	Absent	Glabrous	Glabrous	Glabrous
3	<i>C. malortianus</i>	Multicellular uniseriate and unbranched	Profuse	Profuse	Profuse
4	<i>C. pictus</i>	Absent	Glabrous	Glabrous	Glabrous
5	<i>C. spicatus</i>	Absent	Glabrous	Glabrous	Glabrous
6	<i>C. spiralis</i>	Unicellular, uniseriate and unbranched	Glabrous	Distantly	Profuse
7	<i>C. stenophyllus</i>	Multicellular uniseriate and unbranched	Profuse	Glabrous	Glabrous
8	<i>C. varzearum</i>	Absent	Glabrous	Glabrous	Glabrous
9	<i>C. woodsonii</i>	Absent	Glabrous	Glabrous	Glabrous
10	<i>Hellenias peciosa</i>	Multicellular uniseriate and unbranched	Glabrous	Profuse	Profuse
11	<i>Monocostus uniflorus</i>	Absent	Glabrous	Glabrous	Glabrous
12	<i>Tapeinochilos ananassae</i>	Unicellular, uniseriate and unbranched	Glabrous	Distantly	Glabrous

Key to the species studied based on foliar characters

- 1a. Leaves oval- lanceolate, sessile, lamina 5-8 cm long, leaf margin along with purple line.....*Monocostus uniflorus*
- 1b. Leaves other than oval-lanceolate, petiolate, lamina greater than 8 cm long, leaf margin without any coloured line.....2
- 2a. Leaves elliptic-oblancoelate, leaf sheath papery, brown when mature.....*T. ananassae*
- 2b. Leaves other than elliptic-oblancoelate, leaf sheath fleshy or thick..... 3
- 3a. Leaf sheath purple red or maroon..... 4
- 3b. Leaf sheath green or brown or pink..... 6
- 4a. Leaves linear-lanceolate, pale green on lower side of lamina.....*Hellenia speciosa*
- 4b. Leaves other than linear-lanceolate, maroon colour on lower side of lamina..... 5
- 5a. leaves ovate-elliptic, unicellular hairs present on lower side of lamina..... *C. erythrophyllus*
- 5b. leaves elliptic- obovate, hairs absent on lower side.....*C. varzearum*
- 6a. Trichomes present...7
- 6b. Trichomes absent ...9
- 7a. Trichomes unicellular.....*C. spiralis*
- 7b. Trichomes multicellular8
- 8a. Lamina linear-lanceolate, leaf sheath brown, extended, trichomes only upper side of lamina, absent on petiole.....*C. stenophyllus*
- 8b. Lamina elliptic-oblancoelate, leaf sheath green, not extended, trichomes on both upper and lower side of lamina and petiole...*C. malortianus*
- 9a. Leaf sheath closed with bulging at the tip.....*C. guanaiensis*
- 9b. Leaf sheath closed without bulging at the tip.....10
- 10a. Lamina linear - oblancoelate, margin revolute.....*C. spicatus*
- 10b. Lamina other than linear - oblancoelate, lamina not revolute margin.....11

11a. Lamina elliptic-lanceolate, petiole slightly pinkish along the margin, number of stomata 30-35 per field*C.pictus*

11b.Lamina broad elliptic, petiole green, number of stomata 85-90 per field (25x).....*C.woodsonii*

4. CONCLUSIONS

In higher plants, the taxonomic identity of the species is explained mostly on the basis of floral characters, followed by carpological and foliar characters.

Twelve species belong to four genera in family Costaceae were subjected to the study. In the present study, all the possible parameters coming under macroscopic and microscopic features have been documented and compared. The observations and result revealed that the foliar variations have great taxonomic importance to establish the identity of each species. An artificial key based on foliar characters was prepared and presented where the four genera and twelve species were clearly separated. The data generated in the present study may be useful to detect the identity of a species, even in the absence of flowers. These results point out the significance of foliar characters in systematic as well as species determination.

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