J. Indian bot. Soc. (2024)

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.61289/jibs2024.03.22.139

www.indianbotsoc.org

RESEARCH ARTICLE

Leaf epidermal characterization of selected species of genus *Curcuma* L. (Zingiberaceae)

Seema R.[1*], Madhvi Singh[2], Seshu Lavania[3]

Abstract

The genus *Curcuma* (Zingiberaceae) is known for its medicinal and economic importance. The present study was to examine and establish some interspecific variations among *Curcuma longa* L., *Curcuma aeruginosa* Roxb., *Curcuma amada* Roxb., *Curcuma aromatica* Salisb., *Curcuma caesia* Roxb., *Curcuma ecalcarata* Sivar. & Balach., *Curcuma haritha* Mangaly & M. Sabu, and *Curcuma zedoaria* Roxb. by characterizing the leaf epidermal features using light microscopy (LM) and scanning electron microscopy (SEM) which can be used as a diagnostic tool for species identification. The leaves were amphistomatic with tetracytic stomata more frequently on the abaxial surface. Trichomes were unicellular, non-glandular, conical, and densely distributed on the abaxial surface of *C. aromatica* and *C. haritha,* very few in *C. longa* and *C. aeruginosa.* Stomatal index, stomatal density, and epidermal cell size were maximum in *C. aromatic* and minimum in *C. longa* in the abaxial leaf surfaces statistically significant at $p \leq 0.001$. Correlation analysis showed high positive correlation between stomatal index and density of leaf abaxial surface (r = 0.99), stomatal and epidermal cell density of adaxial and abaxial epidermal surface (r = 0.99, r = 0.95), guard cell length and epidermal cell frequency of adaxial and abaxial epidermal surface (r = 0.92, r = 0.85) among the selected species.

Keywords: Amphistomatic, Curcuma, Epidermis, Tetracytic, Zingiberaceae.

Introduction

The genus *Curcuma* L. belongs to the family Zingiberaceae, composed of 80 species of rhizomatous herb from the Indo-Malayan region, about 40 of which are indigenous to India. *Curcuma L*. is taxonomically a complicated genus which has been divided into two subgenera *Eucurcuma* and *Paracurcuma* based on morphological traits which is still under question (Maknoi *et al*. 2002). Several studies have been already reported on morphological, anatomical and phytochemical aspects of rhizomes of *Curcuma* due to its medicinal and commercial value (Sherlija *et al*. 1998, Choudhury *et al*. 1996 and Seema *et al*. 2022). Despite the medicinal and economic importance of the genus, the information on leaf epidermal micromorphology using SEM is very limited even though some pharmacogenetic, epidermal morphology and essential oil studies of the leaf have been reported (Gogoi *et al*. 2002, Xiao *et al* 2001, Behura *et al*. 2004, Shyam *et al*. 2012 and Seema *et al*. 2015 and 2020). The use of SEM in this study may support and strengthen the results of light microscopy in showing distinct aspects of some diagnostic features important for taxonomic studies (M. Singh *et al*. 2020).

Material And Methods

Plant material

The plant samples were collected from Kannur and Thiruvananthapuram Districts of Kerala. The taxonomic identities of the species studied were determined by comparison with the authentic herbarium specimens deposited at the herbarium of Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Palode,

[1]North East Regional Institute of Education (NERIE-NCERT), Umiam, Shillong.

[2]Department. of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India.

[3]Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India.

***Corresponding Author:** Seema R, North East Regional Institute of Education (NERIE-NCERT), Umiam, Shillong, E-Mail: seeseema2@gmail.com

How to cite this article: Seema, R., Singh, M., Lavania, S. 2024. Leaf epidermal characterization of selected species of genus *Curcuma* L. (Zingiberaceae). J. Indian bot. Soc., Doi: 10.61289/jibs2024.03.22.139

Source of support: Nil

Conflict of interest: None.

Received: 23/02/2024 **Accepted:** 22/03/2024 **Published online**: 25/10/2024

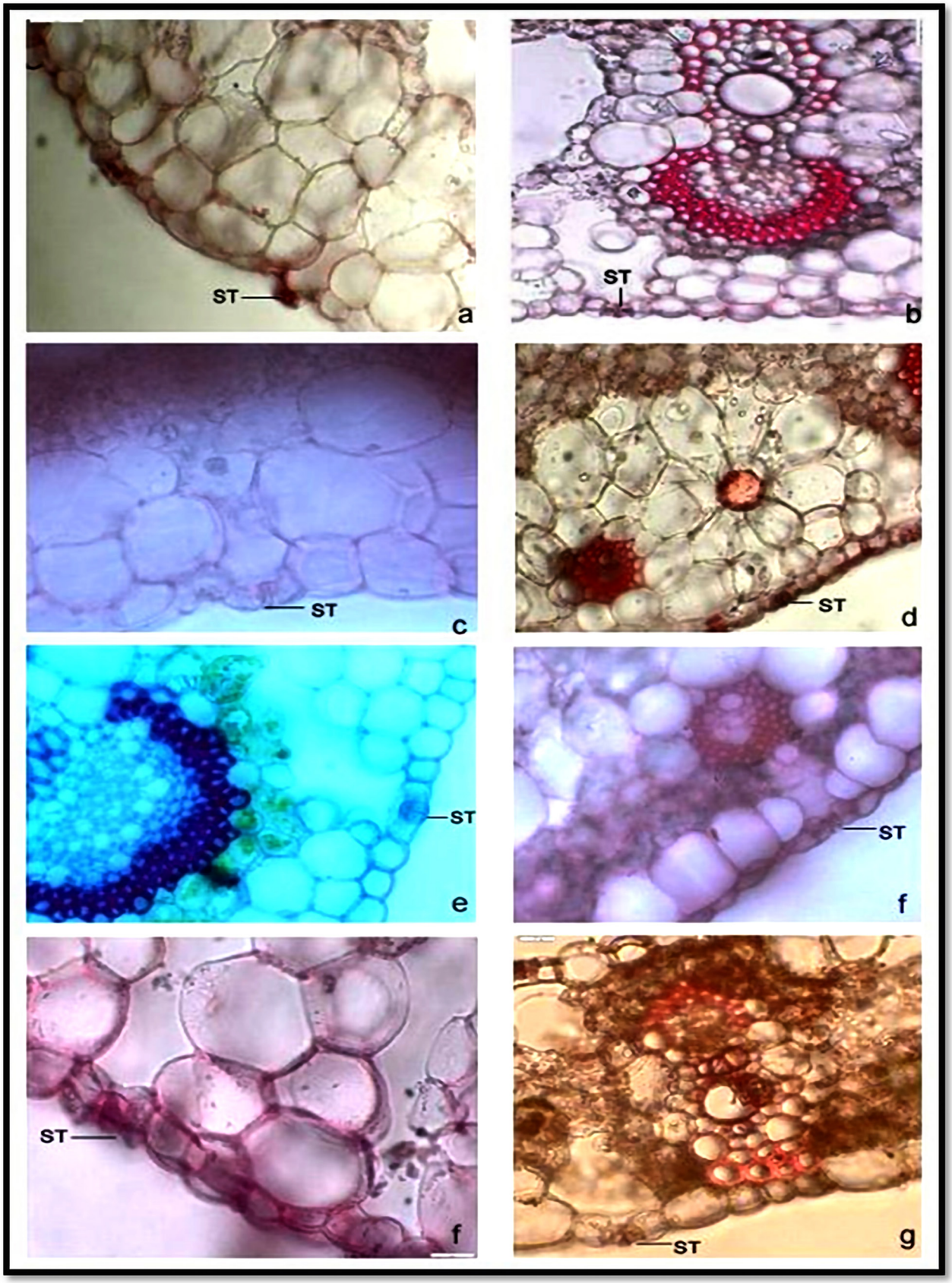

Figure 1: Vertical section of leaf showing stomatal positions under LM. (a) *C. longa;* (b) *C. aeruginosa;* (c) *C. amada;* (d) *C. aromatic*; (e) *C. caesia;* (f) *C. ecalcarata;* (g) *C. haritha;* (h) *C. zedoaria.* ST, stomata

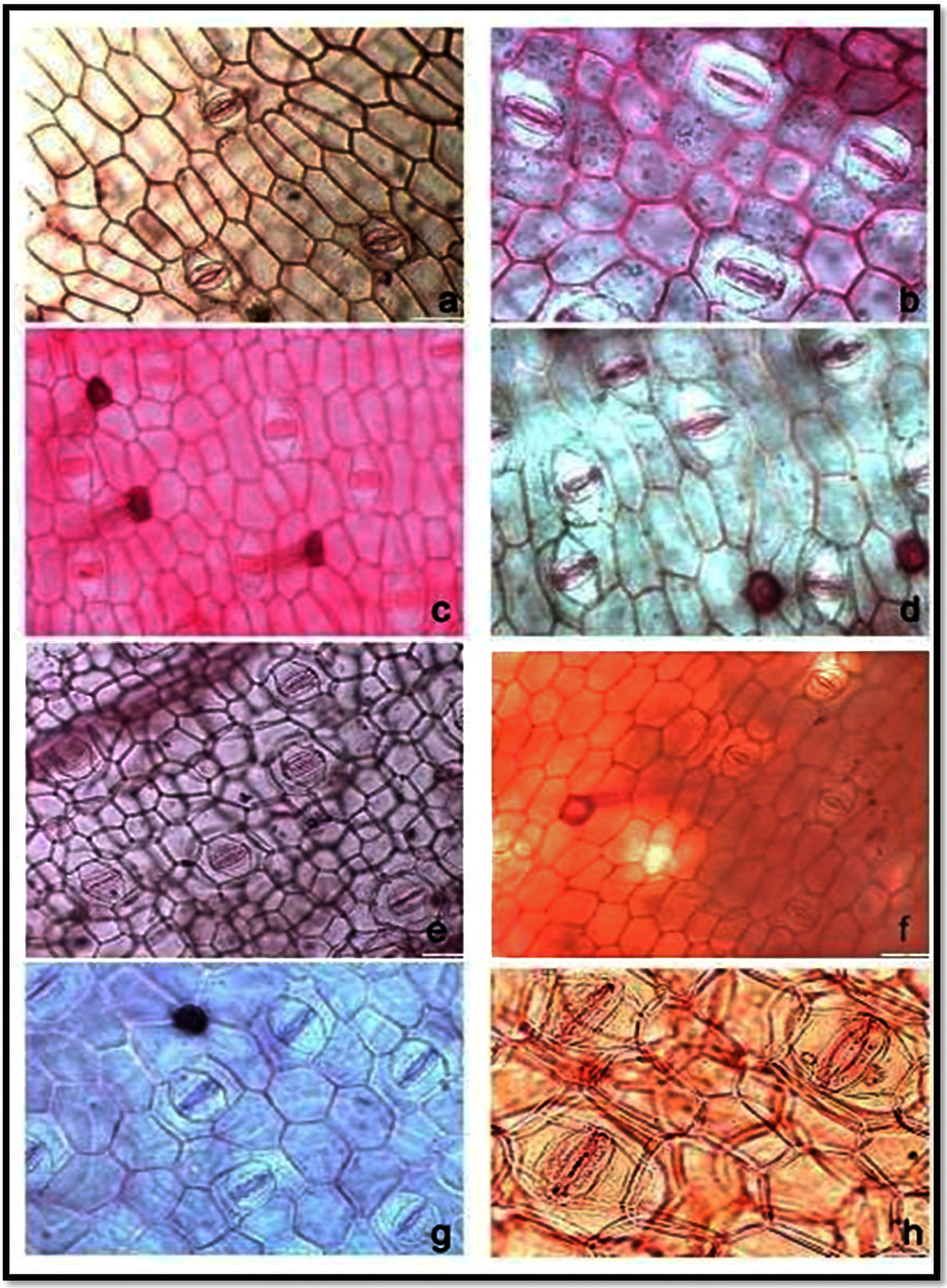

Figure 2: Leaf epidermal peeling from the abaxial surface under LM (a) *C. longa*; (b) *C. aeruginosa*; (c) *C. amada*; (d) *C. aromatica*; (e) *C. caesia*; (f) *C. ecalcarata*; (g) *C. haritha*; (h) *C. zedoaria*. (a- b 100X, h 400X)

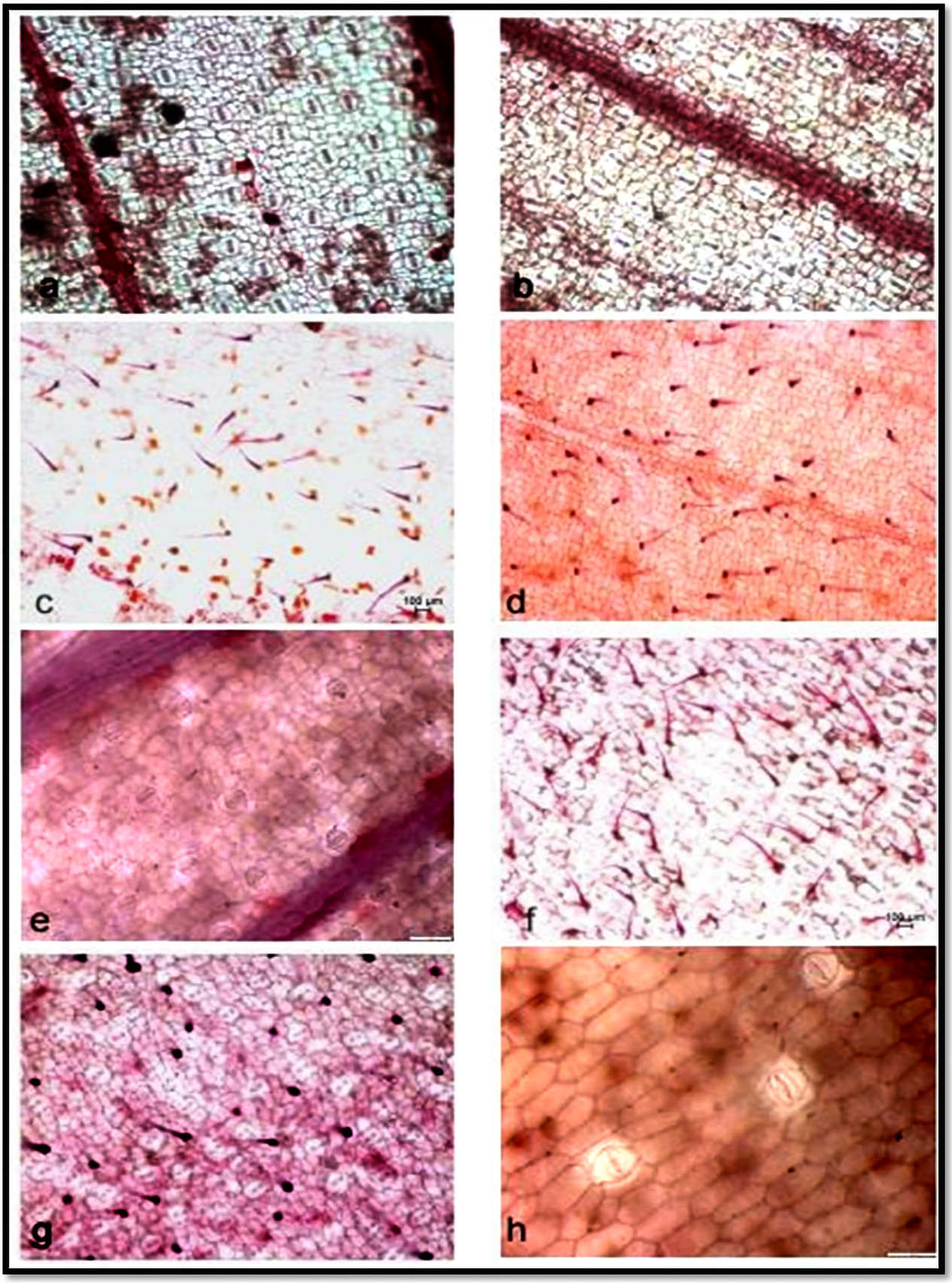

Figure 3: Leaf epidermal peeling from the abaxial surface under LM. (a) *C. longa*; (b) *C. aeruginosa*; (c) *C. amada*; (d) *C. aromatica*; (e) *C. caesia*; (f) *C. ecalcarata*; (g) *C. haritha*; (h) *C. zedoaria*.

Thiruvananthapuram, Kerala (JNTBGRI) and also by the taxonomists. The voucher numbers are *C. longa* (36252, Pathanamthitta, 9-10-1999), *C. haritha* (14576, Thiruvananthapuram, 30-5-1994), *C. aeruginosa* (60668, Thiruvananthapuram, 08-5-2013), *C. zedoaria* (14575, Palode, 30-05-1994), and *C. aromatica* (60664, Palode, 10-01-2013).

Preparation of leaves for Light Microscopy

For all the microscopic studies, samples were taken from the central portion (near the veins) of the fifth matured from the apex side leaf for the consistency of data analysis. The leaf epidermal peels from both adaxial and abaxial surfaces were taken for epidermal characterization. It was then washed and stained with safranin. The light microscopic study was carried out using a brightfield Olympus microscope. The measurements were recorded with a calibrated eyepiece micrometer at 400X and images were taken using a Nikon, HC00L microscope (Figures 1-4).

Preparation of leaves for Scanning Electron microscopy

The fresh leaf samples were collected, washed and cut into pieces of approximately 1 cm^2 each for pretreatment. The samples were fixed in 5% Glutaraldehyde solution for 24hrs, then transferred into NaOH solution of pH=7.5. The leaf pieces were washed thoroughly with distilled water by decantation. The samples were dehydrated by passing through an alcohol series of 10, 30, 50, 70, 90% and finally stored in absolute alcohol. The cleaned samples were air dried and mounted on SEM Aluminium stubs with the help of double-sided adhesive tape, coated by POLARON SC-7640 Sputter coater at 18mA current for 160 seconds in which Gold-Palladium alloy was used as the coating material and scanned using a conventional scanning electron microscope LEO- 430. Photographs were taken at the desired magnification with the help of a secondary electron detector.

Stomatal index

Stomatal index (SI) was calculated according to the formula given by Salisbury (1927) [9] SI= S/ S + E × 100, where S= number of stomata per unit area, E= number of epidermal cells per unit area.

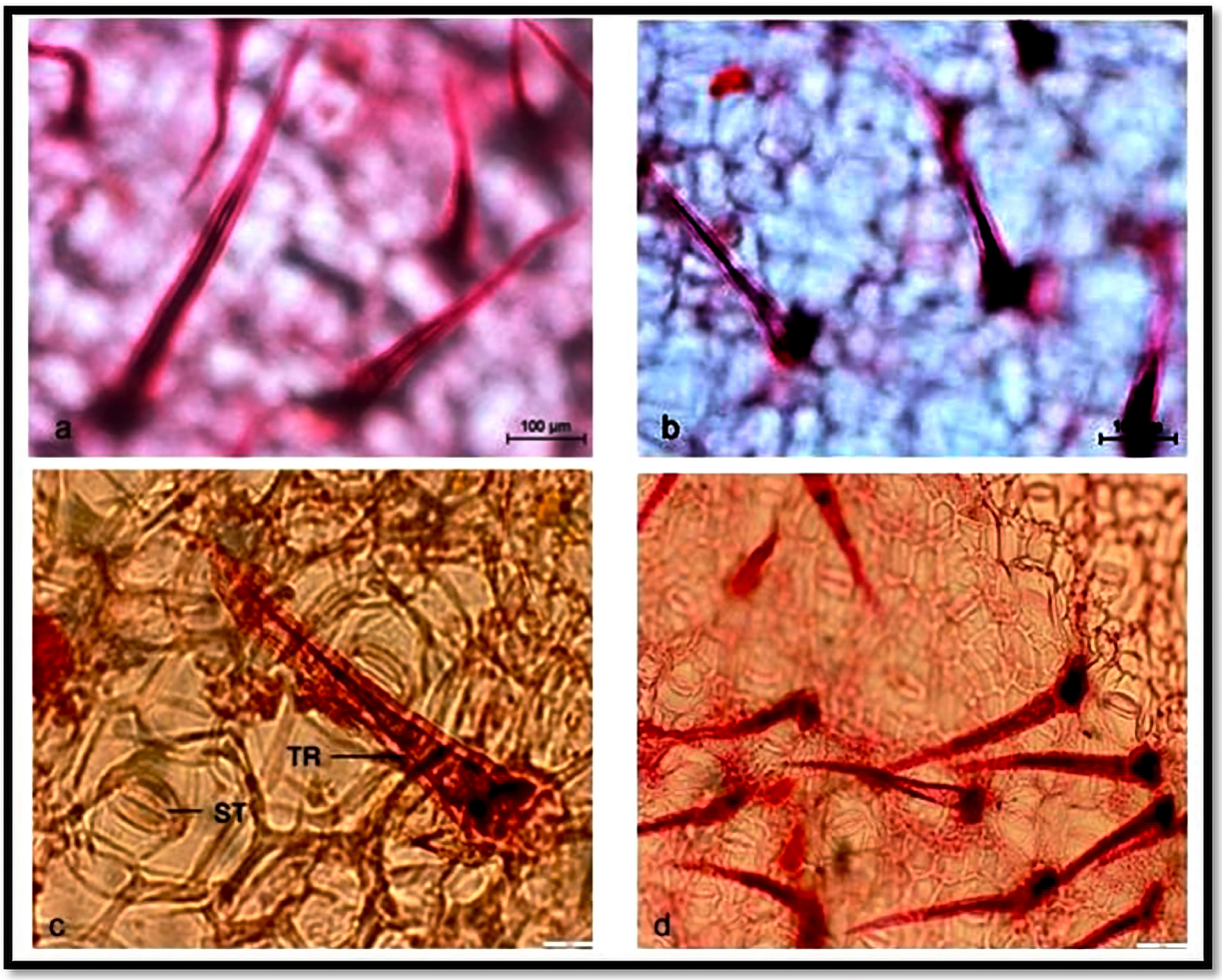

Figure 4: Abaxial Leaf epidermal peeling with trichomes under LM. (a) *C. aromatica*; (b) *C. haritha*; (c) *C. ecalcarata*;(400 X) (d) *C. amada*. ST, stomata; TR, trichome

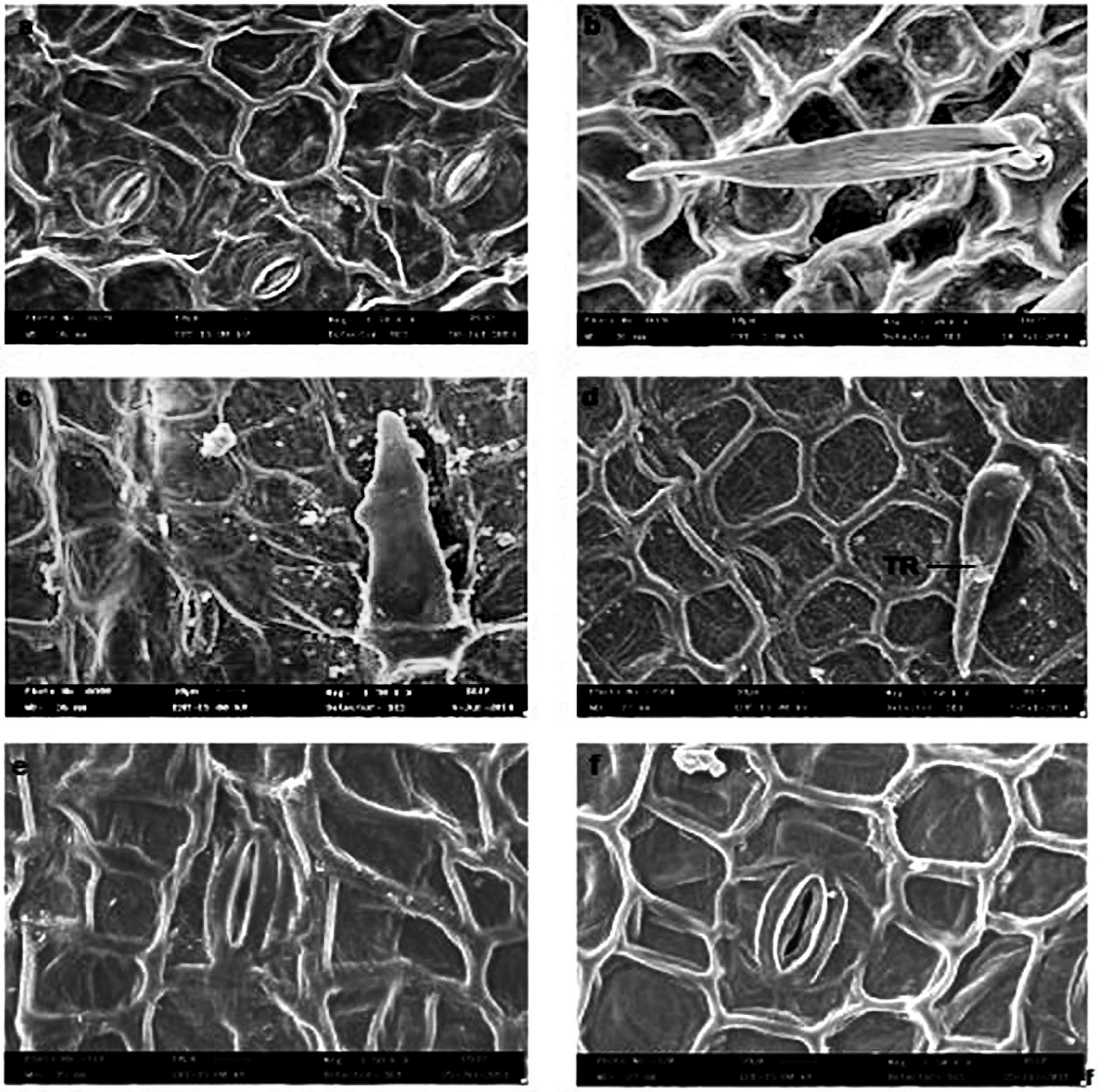

Figure 5: Leaf epidermal morphology; C. caesia under SEM. (a) Abaxial; (b-d) Adaxial. *C. longa* (Market sample); (e) Adaxial; (f) Abaxial. TR, trichome

Results And Observations

Leaf

The leaf is amphistomatic with a single-layered upper and lower epidermis covered by a transparent cuticle. Stomata are tetracytic, oriented parallel to the leaf veins, surrounded by four subsidiary cells, two of them parallel to the guard cells, the remaining two placed polar. The epidermal cells were more or less polygonal in surface view and rectangular in vertical section. The adaxial epidermal cells were larger as compared to the lower epidermal cells. Trichomes are unicellular, non- glandular and conical with a swollen base. The distribution of trichomes showed significant variations among the four species. Trichomes were less in number on the adaxial epidermis and were densely distributed on the abaxial epidermis of *C. aromatica* and *C. haritha* whereas in *C. longa* and *C. aeruginosa* it was found in the leaf margins and very few in the abaxial surface.

Stomata

Stomatal index, stomatal density, and epidermal cell size were maximum in *C. aromatic* and minimum in *C. longa* in the abaxial leaf surfaces statistically significant at $p \leq 0.001$ by one-way ANOVA but no significant difference were observed in the stomatal index, density of adaxial epidermal surface and epidermal cell density of abaxial surface. Guard cell area was found highest in *C. aeruginosa* followed by *C. longa*, *C. aromatica* and *C. haritha*.

Epidermal Cells

Adaxial and abaxial epidermal cell l/w ratio was maximum in *C. aromatica*, followed by *C. haritha*, C. *aeruginosa* & *C. longa* (Figures 5 and 6).

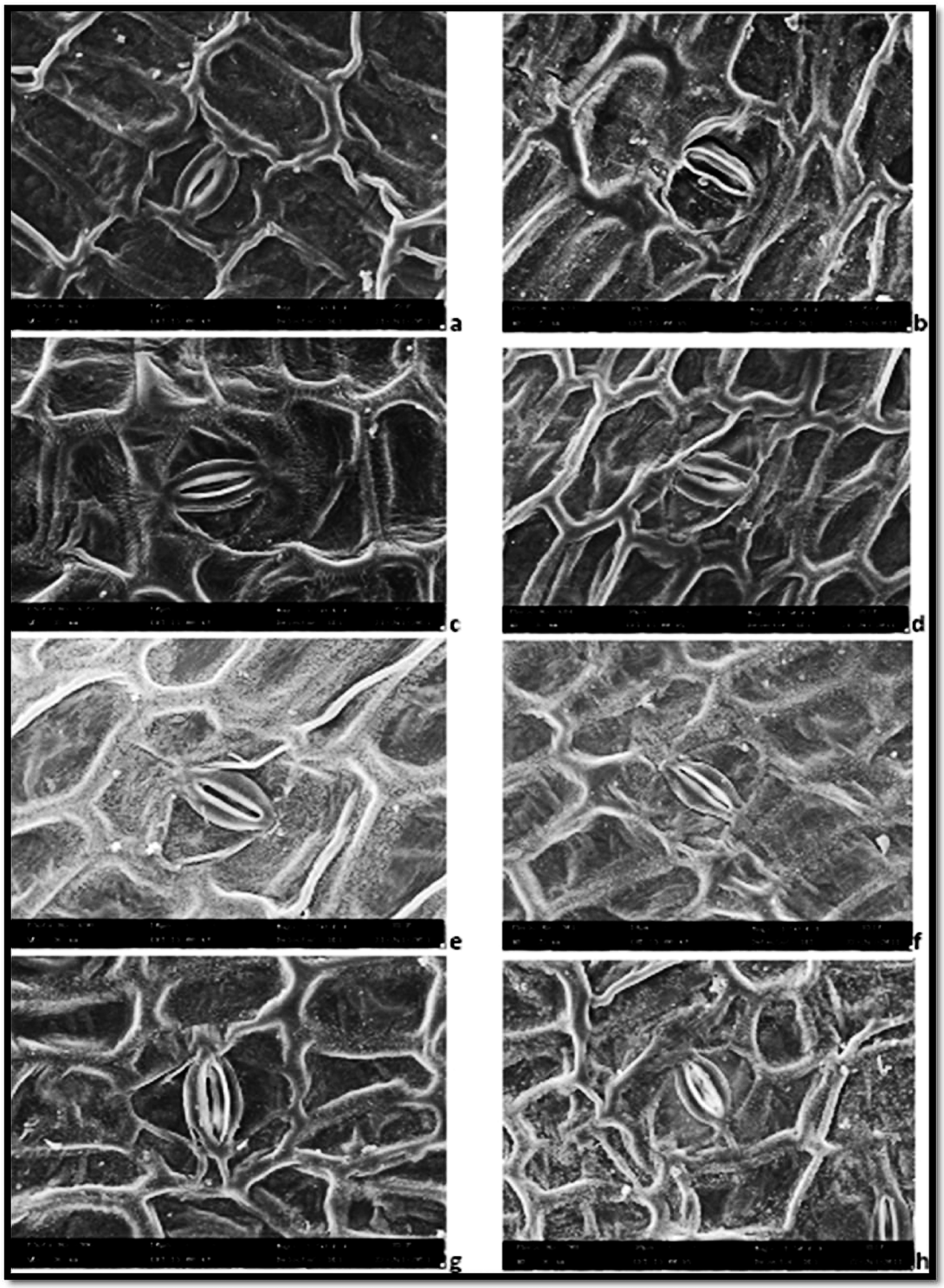

Figure 6: Leaf epidermal features of adaxial surface. (a) *C. oligantha* showing undulated cuticular folds; (b) *C. aeruginosa*; *C. ecalcarata* showing striae radiate; (d) *C. amada*; (e) *C. longa*; (f) *C. haritha*; (g) *C. zedoaria*; (h) *C. aromatica*. Furfuraceous ornamentation in (b), (d), (e) and (f)

Trichome

Trichomes are unicellular, non-glandular and conical with a swollen base. The distribution of trichomes showed significant variations among the eight species investigated. The trichome density was high with uniform distribution on the abaxial epidermal surface of *C. amada*, *C. ecalcarata*, *C. haritha* and *C. aromatica* (Figure 4 a-d) and lesser on the adaxial epidermal surface. In *C. longa* (Figure 3 a), *C.*

olignantha, *C. aeruginosa* and *C. zedoaria* trichomes were observed mostly in the leaf margins and very rarely occurred on the abaxial surface and were absent on the abaxial surface. In *C. caesia* the trichomes were present on the abaxial surface and distributed sparingly (Figure 5 b-d), but it was found absent on the abaxial surface (Figure 5 a Tables 1 and 2). The trichomes of the abaxial epidermis of *C. amada*, *C. ecalcarata*, *C. haritha* and *C. aromatica* were quantified as the density was high there. (Table 3). The length of the trichomes was found maximum in *C. aromatica* (218.2 ± 5.2 µm) followed by *C. ecalcarata* (192.2 ± 7.1µm), *C. haritha* (176.8 ± 7.3 µm) and *C. amada* (164.8 ± 6.2 µm). When statistically analyzed, *C. aromatica* varied significantly from that of *C. ecalcarata* (p≤ 0.05), *C. haritha* and *C. amada* (p≤ 0.001). *C. amada* was found different from *C. ecalcarata* at p≤ 0.05. The trichome density was recorded highest in *C. amada* (982 ±26 mm^2) followed by *C. aromatica* (781 ± 13 mm^2), *C. haritha* (789 ± 13.4 mm^2) and *C. ecalcarata* (393 ± 7 mm^2). All the species showed significant variation at p≤ 0.001 among each other except *C. aromatica* and *C. haritha* which was observed non-significant.

Scanning Electron Microscopic observations

The epidermal cells were rectangular, hexagonal, and polygonal in shape among the different species (Figure 5). In *C. olignantha*, the epidermal cells were more or less hexagonal in the adaxial surface (Figure 5 a). Both adaxial and abaxial surfaces showed rectangular-shaped cells in *C. longa*, *C. zedoaria* and *C. aeruginosa* (Figure 5 b, e, g), *C. longa* (market sample) (Figure 5 e, f). The epidermal cells were polygonal in the adaxial surface (Figure 6 c, d, f, h) as well as in the abaxial surface (Figure 6 b, d, e, f) in *C. ecalcarata*, *C. amada*, *C. haritha*, and *C. aromatica*. In *C. caesia*, the adaxial epidermal cells appeared rectangular whereas the cells were polygonal in the abaxial surface (Figure 5 a). *C. longa* and *C. longa* (market sample) exhibited more or less similar-sized cells on both surfaces under scanning electron microscope whereas in all other species, the size of the epidermal cells was bigger on the adaxial surface in comparison with the cells which appeared smaller in the abaxial leaf surface. In *C. caesia*, the trichome distribution was quite different from all the other species since the trichomes were absent in the lower epidermis but observed in the margins as well as in the upper epidermis. However, the trichomes were less frequent in their distribution (Figure 5 a-d).

The guard cell position was found to be at level with the surrounding epidermal cells in the adaxial surface of all the *Curcuma* species. However, it showed significant variation in the abaxial surface when viewed under SEM. The abaxial surface of *C. olignantha*, *C. ecalcarata*, *C. amada* and *C. aromatica* had slightly depressed or sunken guard cells (Figure 6 d-g) concerning the epidermal cells surrounding the stomatal complex, whereas in *C. longa* and *C. aeruginosa*, *C. haritha*, *C. zedoaria* (Figure 6 a-c, h), *C. caesia* and *C. longa* (market sample) (Figure 5 a, f) the guard cells was in level with the epidermal cell (Table 1).

The cuticular ornamentations were more prominent on the adaxial epidermal cells in all the investigated species of *Curcuma* which showed cuticular folding with epicuticular wax depositions. Thus, more variations were observed in the adaxial surface than the abaxial surface (Figure 6). Under SEM view both surfaces showed a uniform undulating cuticular folding with periclinal and anticlinal walls with shallow depressions on their surface in all the species (Table 2).

Epicuticular wax ornamentations were observed at higher magnification. It appeared as striations, perpendicular striae radiate, on the adaxial surface of *C. ecalcarata* (Figure 6 c) whereas in *C. longa, C. aeruginosa* and *C. haritha* undulating cuticular was widely spread with minute furfuraceous ornamentation (Figure 6 b, e, f). In *C. zedoaria* and *C. aromatica* the furfuraceous ornamentation was less widely spread on the undulating cuticular folds in the adaxial epidermal surface (Figure 6 g, h).

Further, the epidermal features observed, measured, and quantified under LM were analyzed statistically for their significance. The quantified measurements of LM data and

Table 1: Leaf stomatal features of different *Curcuma* species under LM

S. No.	Name of Species	Type of stomata	Guard cell Length (µm)	Guard cell Width (µm)	Ratio (l/w) Guard cell	Adaxial surface			Abaxial surface		
						Stomatal Density (mm^2)	Epidermal Cell density (mm^2)	Stomatal index (%)	Stomatal Density (mm^2)	Epidermal Cell density (mm^2)	Stomatal Index (%)
1	C. longa	Tetracytic	42.6 ± 0.39	10.8 ± 0	3.94±0.04	13.3 ± 0	635± 7.6	2.05±0.02	46.5±1.52	725 ± 4.8	6.03 ± 0.2
2	C. aeruginosa	Tetracytic	42.6 ± 0.29	10.44 ± 0.24	4.08±0.14	15± 0.9	667 ± 8.9	2.15±0.14	67 ± 3.53	730±6.98	8.39 ± 0.4
3	C. amada	Tetracytic	40 ± 0.63	10.8 ± 0	3.7±0.06	13.9± 0.5	776 ± 11	1.76±0.07	58.4± 2.02	849.4±16.2	6.48 ± 0.26
4	C. aromatica	Tetracytic	39 ± 0.6	10.9 ± 0.4	3.57±0.14	15.3 ±1.1	609 ± 7.9	2.43±0.15	75.7 ± 3.9	722± 8.6	9.5 ± 0.49
5	C. caesia	Tetracytic	32.5 ± 0.44	10.8 ± 0.3	3.08±0.13	16.6 ±1.3	664 ± 12.8	1.96±0.1	57.7 ± 3.1	722±12.4	7.4±0.33
6	C. ecalcarata	Tetracytic	36.9 ± 0.44	10.44 ± 0.24	3.6±0.15	13.9±0.6	672 ± 9.5	2.04±0.11	43.17 ± 2.5	743±12.5	5.45 ± 0.2
7	C. haritha	Tetracytic	37.1 ± 0.52	10.8 ± 0	3.43±0.05	14 ± 0.6	574 ± 5.6	2.36±0.09	56 .4 ± 2.9	706 ± 9.5	7.38 ± 0.32
8	C. zedoaria	Tetracytic	41.5 ± 0.55	10.8 ± 0	3.85± 0.05	25 ± 1.4	814 ± 15.7	2.97±0.19	93 ± 2.54	724±15.1	11.5 ± 0.46

Table 2: Leaf epidermal cell features of different Curcuma species under LM

S. No.	*Name of species*	*Adaxial cell Length (µm)*	*Adaxial cell Width (µm)*	*Ratio (l/w) (Adaxial)*	*Abaxial cell Length (µm)*	*Abaxial cell Width (µm)*	*Ratio (l/w) (Abaxial)*
1	C. longa	47.5±1.7	37.4±1.2	1.27± 0.6	37±0.9	28.4±09	1.32±0.03
2	C. aeruginosa	40.3±1.05	30.6± 1.23	1.34± 0.07	31±1.4	21.2±0.9	1.46±0.07
3	C. amada	39.2±0.9	20.9± 0.9	1.92 ± 0.12	32.7±0.9	19.8±0.6	1.66 ± 0.06
4	C. aromatica	51.4±2.3	23.4±1.23	2.25± 0.17	49.3±2.7	20.5±0.7	2.40±0.16
5	C. caesia	36.4±1.4	21.2± 0.9	1.74± 0.1	29.2±0.84	19.08±0.74	1.55 ± 0.9
6	C. ecalcarata	45 ± 1.9	27 ± 1.2	1.7± 0.1	33.48 ± 1.1	21.4 ± 0.9	1.6 ± 0.09
7	C. haritha	49.7±1.05	27±1.23	1.84±0.07	37±0.7	23±0.8	1.6±0.04
8	C. zedoaria	46.8±1.8	27±1.23	1.75 ±0.08	36±1.2	25.2±1.4	1.46 ± 0.07

Table 3: Leaf trichome features of selected species of Curcuma under LM

S No.	Name of Samples	Trichome density	Mean Size of Trichome (µm)
1	C. ecalcarata	393 ± 7	192.2± 7.1
2	C. amada	982 ± 26	164.8± 6.2
3	C. haritha	789 ± 13.4	176.8± 7.3

SEM observations were used in the characteristic analysis of different *Curcuma* species (Table 2).

The correlation analysis was performed among stomatal index, frequency, trichome density and size. The stomatal index and stomatal frequency of both adaxial and abaxial surfaces exhibited high positive correlation r = 0.89 and 0.84. Adaxial stomatal index and stomatal frequency showed a correlation value of r = 0.86, similar relationship was also obtained in the case of abaxial surface with a value of r = 0.96. Adaxial epidermal cell length had a positive correlation with the abaxial surface (r = 0.82). Adaxial epidermal cell width also exhibited a positive correlation (r = 0.84) with that of the abaxial surface. Trichome density and size of *C. amada*, *C. ecalcarata*, *C. haritha* and *C. aromatica* were quantitative and examined due to their dense distribution in the lower epidermis. Statistical analysis showed a significant difference in the trichome density but not in their size. Further, trichome density and size showed a high negative correlation among the four species (Table 3). However, the trichome length was positively correlation with stomatal index (r = 0.82), density (r = 0.87) and epidermal cell length of the abaxial surface (r = 0.85). Statistical results of Bartlett's test for equal variance from one-way ANOVA (p≤0.05) of the quantitative parameters of leaf epidermis analyzed among the *Curcuma* species are shown in Table 4.

Statistical Analysis

Highly positive correlation between stomatal index and the stomata frequency of the abaxial surface (r = 0.99), stomatal and epidermal cell frequency of adaxial and abaxial surface (r = 0.99, r = 0.953), guard cell length and epidermal cell frequency of adaxial and abaxial surface (r = 0.92, r = 0.85).

Table 4: Statistical results of ANOVA (p≤0.05) of the quantitative parameters of leaf epidermis analysed among the Curcuma species

S. No	*Characters*	*Significant (p≤0.05)*
1	Abaxial cell length (µm)	Significant
2	Abaxial cell width (µm)	ns
3	Adaxial cell length (µm)	ns
4	Adaxial cell width (µm)	ns
5	Ratio abaxial length to width	Significant
6	Ratio adaxial length to width	Significant
7	Stomatal density (adaxial)	ns
8	Stomatal density (abaxial)	Significant
9	Stomatal index (%) (adaxial)	Significant
10	Stomatal index (%) (abaxial)	Significant
11	Guard cell length (µm)	Significant
12	Guard cell width (µm)	ns
13	Ratio guard cell length to width	Significant
14	Trichome density (%)	Significant
15	Trichome length (µm)	ns

ns, non-significant

Discussions And Conclusions

In the SEM analysis wax ornamentations were visible only under SEM with a higher magnification. It appeared as striations, perpendicular striae radiate, in the adaxial surface of *C. ecalcarata* (Figure 6 c) whereas in *C. longa, C. aeruginosa* and *C. haritha* undulating cuticular was widely spread with minute furfuraceous ornamentation (Figure 6 b, e, f). In *C. zedoaria* and *C. aromatica* the furfuraceous ornamentation was less widely spread on the undulating cuticular folds in the adaxial epidermal surface (Figure 6 g, h).

Further, the epidermal features observed, measured, and quantified under LM were analyzed statistically for their significance. The quantified measurements of LM data and SEM observations were used in the characteristic analysis of different *Curcuma* species. The leaf surface

micromorphological characterization of the examined species is useful as a species identification tool. The interspecific variations have taxonomic and morphological significance.

Acknowledgement

The authors are thankful to The Birbal Sahni Institute for Paleobotany, Lucknow, for providing SEM facilities. One of the authors Dr. Seema R. is thankful to the taxonomists of TBGRI, Thiruvananthapuram, Kerala for the authentication of plant specimens.

References

Velayudhan KC, Muralidharan VK, Amalraj VA, Gautam PL,Mandal S and Dinesh Kumar. (1999). *Curcuma* Genetic Resources. Scientific Monograph No. 4. New Delhi: National Bureau of Plant Genetic Resources.

Maknoi C and Sirirugsa P. (2002) Notes on the infra-generic classification of the genus *Curcuma* L. Poster abstract. 12th Flora of Thailand meeting, Forest herbarium, Phaholyothin Road, Chatuchak, Bangkok 10900, Thailand.

Sherlija KK, Remasree AB, Unnikrishnan K and Ravindran PN. (1998). Comparative rhizome anatomy of four species of Curcuma. *Journal of Spices & Aromatic Crops* 7: 103–109.

Choudhury SN, Ghosh AC, Saika M, Choudhury M and Leclercq PA. (1996). Volatile oil constituents of the aerial and underground parts of *C. aromatica* Salisb. from India. *Journal of Essential Oil Research* 8: 635–638.

Gogoi R, Bokolial D and Das DS. (2002). Leaf epidermal morphology of some species of Zingiberaceae. *Plant Archives* 2: 257–262.

Xiao XM, Xia WJ, Qin SY, Li JL, Fang QM, Shu GM and Su ZW. (2001). Pattern of recognition of stereoscopic features of the leaf epidermis of medicinal Curcuma plants in China by image analysis. *Zhongguo Zhong Yao Za Zhi* 26: 523–528.

Behura S and Srivastava VK (2004) Essential oils of leaves of Curcuma species. *Journal of Essential Oil Research* 16:109–110.

Shyam Prasad M., Anju P. Ramachandran, Harimohan Chandola, Harisha C. R., Vinay J. Shukla. (2012). AYU Pharmacognostical and phytochemical studies of *Curcuma neilgherrensis* (Wight) leaf - A folklore medicine **33**: 2.

Salisbury. (1927) .On the causes and ecological significance of stomatal frequency, with specia reference to the wood-land flora. *Phil.Trans.R.Soc.*216B, 1- 65

Seema, R. and Seshu Lavania. (2015). Histochemical localization of curcumin and its

significance in chemotypic characterization of selected species of *Curcuma L*., *Industrial Crops and Products* 65: 175- 179.

Seema, R., Anil Kumar K. S., Seshu Lavania. (2020). Histochemical localization and chromatographic analysis of leaf essential oils of selected species *Curcuma L*., *Plant Archives* 20 (1)1: 1247- 1251.

Seema, R. and Seshu Lavania. (2022). Rhizome extract of Curcuma longa L. exhibit mitodepressive effect, but no genotoxicity , *Journal of Indian Botanical Society* 102 (1): 67- 72.

Singh M., Vimala Y., Lavania S. & D. Verma. (2020) Leaf epidermal features in relation to taxonomy of some species of *Bulbophyllum* (Orchidaceae) from Northeast India. *Rheedea* 30(4): 427–443.