



Foliar Micromorphological and Architectural Studies of Glory Lily (*Gloriosa superba* L.) – An Important Medicinal Plant

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ABSTRACT

Foliar micromorphological study of plants describes the distinct structure and functions of foliar tissues. The structural analysis of *G. superba* epidermal tissues and leaf architecture revealed the stomatal type, morphology and their orientation, cuticular wax deposition, crystal arrangement and venation pattern. The stomatal density was observed maximum on abaxial epidermis. The epidermis contained ordered stomatal pattern with anomocytic stomata. The stomatal density and stomatal index was 12.0 and 29.0 respectively. The parallel lower order veins and perpendicular higher order veins form a grid-like network of venation pattern in *G. superba*. Con-vallaria type of cuticular wax was observed. The results of the foliar features could be applied in systematic studies and also to predict the environmental factors responsible for the development of certain leaf parameters.

Keywords: *Gloriosa superba*; Stomatal morphology; Crystals; Cuticular Wax

1. INTRODUCTION

Gloriosa superba L. (Liliaceae) is one among the seven Upavishas in the Indian traditional systems of medicines and an endangered medicinal plant species (Singh *et al.*, 2013). It is a perennial woody climber, grows up to the height of 5 m, native of the Africa and

Southeast Asia, and growing naturally throughout the India, Sri Lanka, Malaysia, Burma and United States (Jayaweera, 1982).

It is commonly known as Climbing-lily, Creeping-lily, Flame-lily, Glory-lily and Tiger claw. *Gloriosa rothschildiana*, *G. simplex*, *G. virescens*, *G. abyssinica*, *G. carsonii*, *G. minor*, *G. lutea*, *G. baudii* are the synonyms of *G. superba* (Rajak and Roy, 1990). The long, thick and wiry stems bear sessile, lanceolate alternate leaves with spiral tendrils. The roots are fleshy and tuberous. Flowers are large, solitary, twisted and crisped with six recurved petals and blooming during the months of November to March. The flower emerges as yellow bud and gradually changes to yellowish red and finally becomes deep scarlet on maturity. Fruits are capsules, which contain numerous warty and compressed seeds (Banu and Nagarajan, 2012; Geetanjali *et al.*, 2012).

Traditionally the tuberous root with sesame oil is used to treat arthritis, intestinal worms, bruises, infertility, skin problems, impotence, gout and inflammation, ulcers, bleeding piles, leprosy and snakebites (Tiwari and Yadav, 2003; Sahu *et al.*, 2010).

Phytochemical characterization of *G. superba* reveals the presence of colchicine (active alkaloid), gloriosine (Gooneratne, 1966; Angunawela and Fernando, 1971), lumicolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine etc. (Chulabhorn *et al.*, 1998). The tuber and seeds are reported to contain higher quantity of colchicine, colchicoside, superbine, gloriosine (Jain and Suryavanshi, 2010), 3-demethyl-N-deformyl-N-deacetylcolchicine, luteolin, tannins, superbine, benzoic and salicylic acid, sterols, silosterol and flucoside (Chitra and Rajaman, 2010; Ashokkumar, 2015).

Due to the presence of these phytochemicals, *G. superba* exhibits various pharmacological activities such as antithrombotic (Kee *et al.*, 2008), anti-venom property (Haroon, 2008), anti-inflammatory (Jomy *et al.*, 2009), antimicrobial (Hemaiswarya, 2009), anticancer (Reuter, 2010), anthelmintic (Pawar, 2010), hepatoprotective (Mohandass, 2011) and antioxidant activities (Amudha and Shanthi, 2011).

The imprudent harvesting of *G. superba* for colchicine contents in tubers and poor seed setting resulted in scarcity of the species under natural conditions and pushed the plant into threatened category (Ade and Roy, 2009; Mishra and Kotwal, 2009).

The literature survey revealed that the plant has not been explored in the field of foliar micromorphological evaluation. Based on this axiom, we have evaluated the foliar micromorphological and architectural parameters of the endangered medicinal plant *Gloriosa superba* L.

The leaf epidermal characters such as morphology and patterns of stomata, size and shape of the cells and cell wall undulations are widely used in systematics of monocot groups (Prat, 1961; Tateoka *et al.*, 1959; Metcalfe, 1960; Ellis, 1979; Stace, 1984). The aim of this study was to determine the foliar micromorphological and architectural parameters such as stomatal pattern, venation pattern, density of stomata, vein islets and veinlet terminations of *G. superba* leaves.

2. MATERIALS AND METHODS

2. 1. Collection and identification plant material

The disease free healthy plants of *Gloriosa superba* were collected during the months of November to April, 2016 from coastal areas of Puducherry (India) and identified with the help

of Gamble flora (Gamble, 1921). Plants were randomly selected and the required leaf samples were collected at third to seventh leaves from the base with sterilized scissor (Fig. 1). Fresh leaves were used for qualitative as well as quantitative micromorphological and leaf architectural studies.



Fig. 1. Leaves samples collected from the selected plant.

2. 2. Foliar micromorphological studies

Experiments were conducted to evaluate the micromorphological features of *G. superba* leaves under natural environment. The foliar micromorphological parameters such as orientation of stomata and their types, morphology, density, distribution and stomatal index were studied through paradermal sections, which were obtained manually by standard method (Johansen, 1940).

2. 3. Evaluation of venation pattern and vein density

For venation pattern, vein density and crystal density study, the leaves were excised and fixed primarily in formalin acetic acid alcohol (FAA in the ratio of 1:1:3) solution. The fixed leaves were cleared in 70% ethanol (v/v) until chlorophyll was completely removed (12-24 h), bleached with 5% (w/v) NaOH for 24-48 h, rinsed three times in distilled water and allowed to remain in saturated Chloral hydrate solution for 24-48 h (Sass, 1940). The cleared leaves were used for the study of venation pattern, vein-islets and veinlet terminations and crystal arrangement and their morphology.

The materials were stained with 1% (v/v) safranin (Loba chemie, India) aqueous solution for 3-5 min, the excess stain was removed and then mounted in water, examined under photomicroscope (Labomed iVu 3100, USA) and analyzed using software Pixelpro. These micrographs with different magnifications were used for identification of micromorphological and leaf architectural parameters. Magnifications of the figures were indicated by the scale-bars.

2. 4. Statistical analyses

The data for various parameters like stomatal density, frequency and stomatal index were calculated by the method suggested by Salisbury (1932). Types of stomata have been described by following the classification and terminology as suggested latest by Croxdale (2000) and Prabhakar (2004). The terminology adopted for venation pattern was of Hickey and Wolfe (1975). The statistical analyses were performed by ANOVA using SPSS version 16 (SPSS Inc., Chicago, USA). The significance of differences among mean values was calculated by Duncan's multiple range tests at $P < 0.05$ and the results were presented as mean \pm standard deviation (SD) of three experiments.

3. RESULTS AND DISCUSSION

The epidermal morphology has been used in phylogenetic considerations of species (Chandra *et al.*, 1969). Foliar micromorphological characteristics of a species assist in authentication of foliar drugs in the field of pharmacognosy and these parameters are reported to serve as biomarkers (Gohil *et al.*, 2007). The foliar micromorphological and architectural parameters evaluated in the present study were (i) Stomatal density, (ii) Stomatal index, (iii) Venation pattern and (iv) Crystals morphology. Quantitative stomatal features of *G. superba* are presented in Table 1.

In horticulture and agriculture, leaf surface micromorphology is extensively studied to determine the spread and deposition of foliar spray (Boize *et al.* 1976; Gudim *et al.* 1976). Absorption of herbicides and growth promoting hormones in horticultural varieties is determined by the micromorphological characteristics of the leaves such as cuticle (epicuticular wax), position and angle of leaves, density of stomata and trichomes (Hess 1985; Wanamarta and Penner 1989).

3. 1. Foliar micromorphological studies

3. 1. 1. Epidermal surface

The epidermal cells were large in size, elongated and compactly arranged. The cuticular striations were thick and the undulated anticlinal walls were observed through the paradermal section. The epidermal layers form a boundary between the external environment and internal tissues of the plant. The epidermis protects the plant against water loss, mediates the rate of gas exchange and secretes metabolic compounds (Agbolade *et al.*, 2011). The structural features of the epidermal cells are species specific (Metcalf and Chalk, 1979). The structural stiffness of monocot leaves was due to the inherent folding and curling of epidermal cells (Niklas and Paolillo, 1997; Wiedemann and Neinhuis, 1998).

3. 1. 2. Stomatal pattern and density

The number of stomata per unit area was found maximum on abaxial epidermis than the adaxial epidermis. Similar reports are available in number of monocot grass species such as *Setaria viridis* (Sanyal *et al.*, 2006). Predominantly anomocytic stomata with indistinct subsidiaries (Fig. 2B) were observed in *G. superba*. Clear costal and intercostals regions were distinguished. Stomata pattern was ordered, distributed in intercostals regions and rarely observed on coastal regions of lamina, all the stomata were facing same direction but space between the stomata was varied (Fig. 2A). Single subsidiary was shared by 3-4 stomata when they were closely spaced. The epidermal cells surround the stomata variations in the length. The stomatal density and stomatal index of *G. superba* was 12.0 and 29.0 respectively (Table 1).

Table 1. Quantitative values of stomatal density and stomatal index in *G. superba*.

Field No.	Stomatal density (Mean±SD)	Stomatal Index (SI) (Mean±SD)
1	10.0±0.14 ^a	27.0±0.29 ^b
2	11.0±0.22 ^b	25.0±0.13 ^a
3	13.0±0.10 ^d	32.0±0.21 ^e
4	10.0±0.27 ^a	30.0±0.19 ^d
5	14.0±0.13 ^e	33.0±0.11 ^e
6	13.0±0.10 ^d	25.0±0.14 ^a
7	12.0±0.25 ^c	27.0±0.19 ^b
8	13.0±0.19 ^d	29.0±0.13 ^c
9	11.0±0.11 ^b	30.0±0.18 ^d
10	13.0±0.15 ^d	32.0±0.24 ^e
Mean	12.0±0.18 ^c	29.0±0.12 ^c

Note: Mean separation was analyzed using SPSS software (ver. 16.0), the values represented in corresponding column followed by same letters are not significantly different according to DMRT at $P < 0.05$.

Type and number of stomata are intrinsic characteristics of a particular species at any geographic level (Carpenter and Smith, 1975), but due to environmental factors the shape and structure were modified (Garbutt *et al.*, 1990; Casson and Gray, 2008). Geisler *et al.* (2000) reported that the meristemoid mother cells situated near to the guard cells determine the space between stomatal apparatus. This parameter could be used to identify the growth and developmental stages of plants.

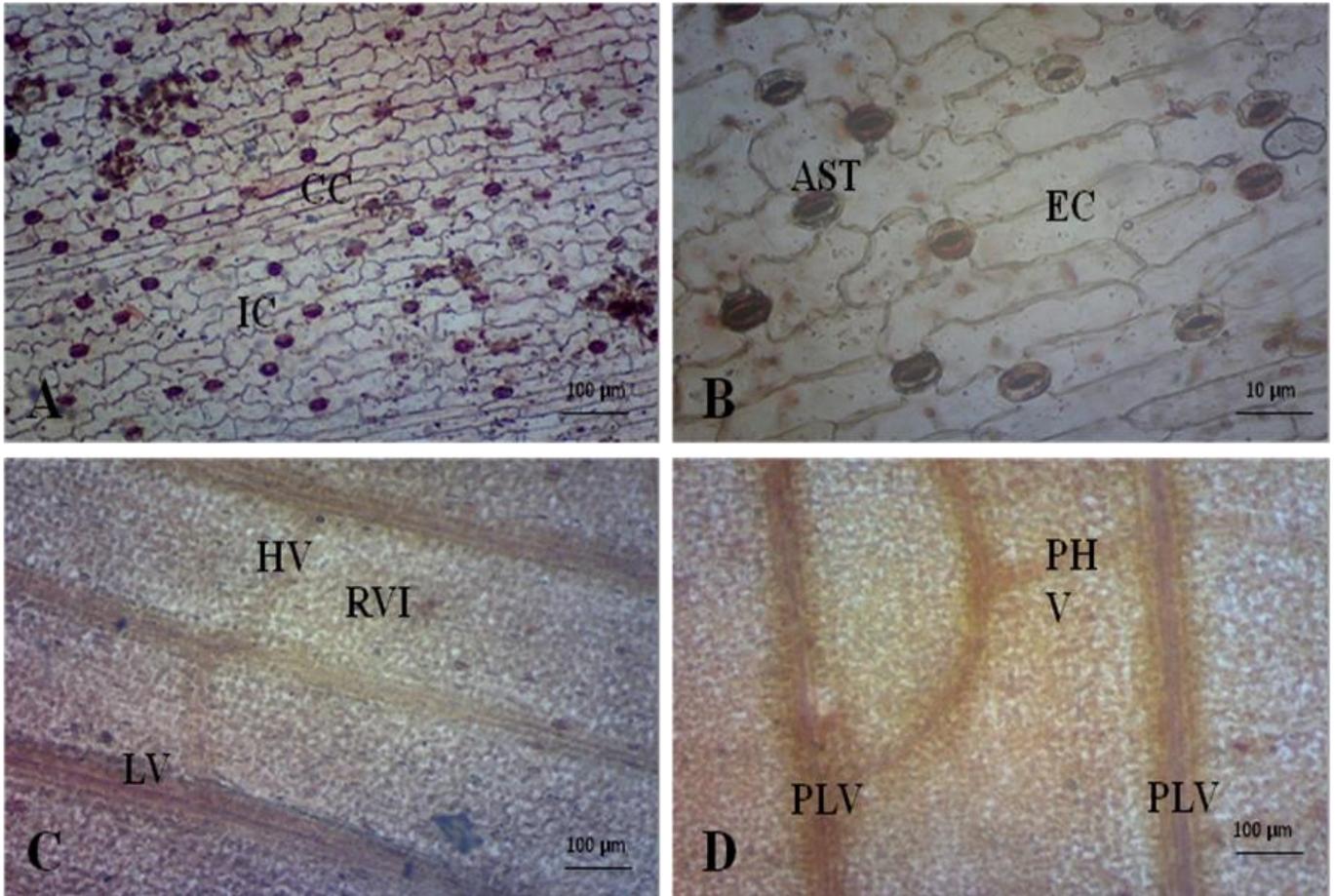


Fig. 2. Pattern of stomata and veins in *Gloriosa superba*.

A - Stomatal distribution and density in abaxial epidermis
(CC – Costal cells, IC – Intercoastal Cells).

B – Paradermal section of stomatal distribution – magnified view
(AST – Anomocytic Stomata, EC – Epidermal Cells)

C – Parallel venation pattern in *G. superba*
(LV- Lower order vein, HV – Higher order vein, RVI – Rectangular Vein Islet).

D – Magnified view of venation pattern
(PLV- Parallel lower order vein, PHV – Perpendicular higher order vein).

The regular pattern of stomata and pavement cells in alternating cells files are characteristic feature of monocotyledonous plants (Croxdale, 2000). The highest stomatal density in the abaxial epidermis and lowest on the adaxial surface was reported to prevent excess water loss, because the abaxial surface is less exposed to heating (Martin and Glover, 2007). The absence of stomata on coastal cells (veins) could help in preventing water loss and also to protect the photosynthetically active palisade mesophyll cells which are associated with vascular bundles (Casson and Gray, 2008).

3. 1. 3. Venation pattern and density

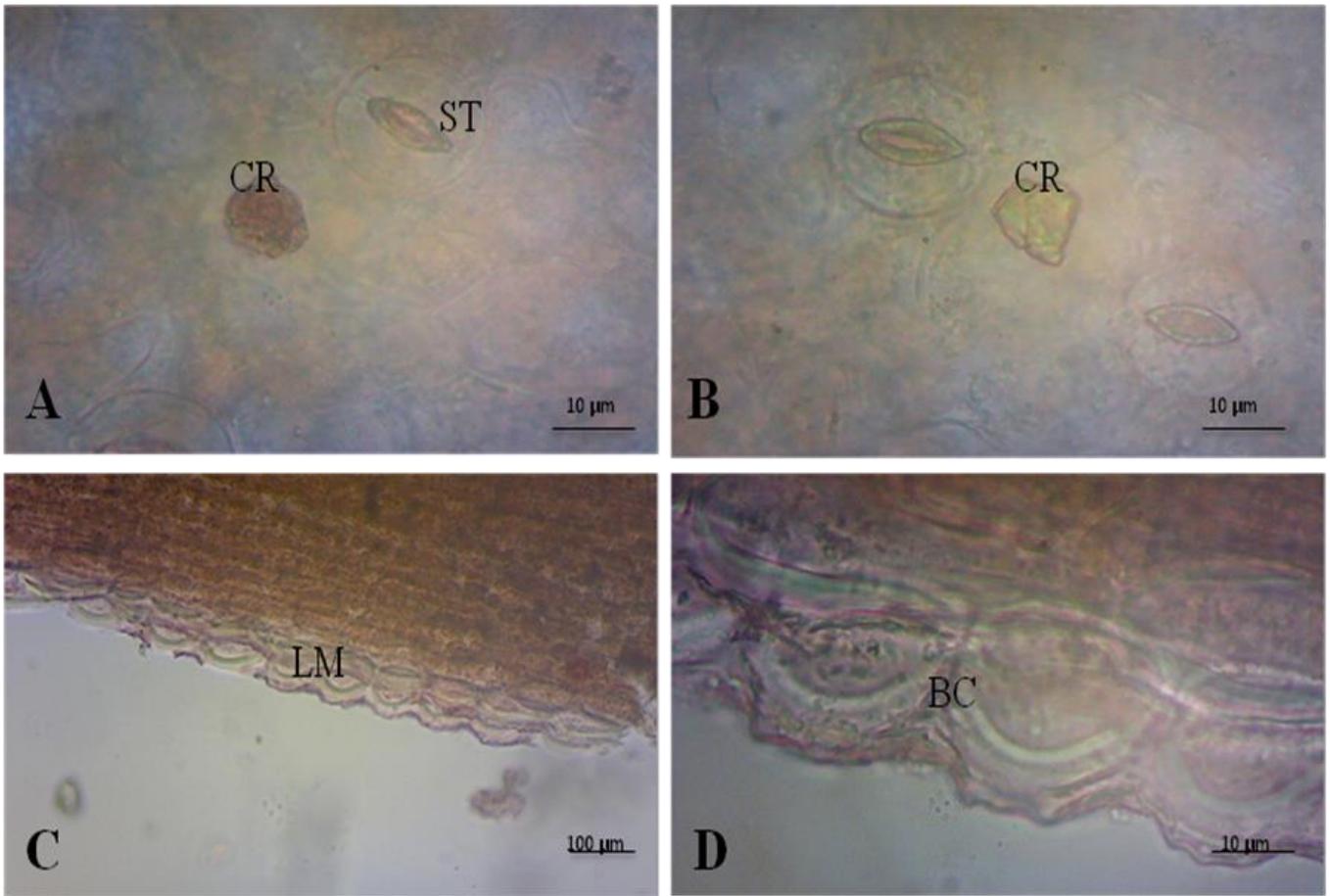


Fig. 3. Structure and distribution of crystals and structure of leaf margin of *G. superba*.

A and B – Distribution and morphology of Calcium oxalate crystals on abaxial epidermis.

C – Leaf margin with epicuticular wax (LM – Leaf Margin).

D – Magnified Bulliform cells (BC – Bulliform Cells)

Visually the leaves possessed parallel veins and tips ending in spiral tendrils which are reported to be used for climbing. Vein clearing study revealed that the mid vein was prominent and the lower order veins run parallel to the full length of lamina and these were interconnected by cross linked by higher order veins which formed grid-like network in *G. superba* through light microscopic analysis. Rectangular vein-islets were observed (Fig. 2C). The higher order veins are perpendicular to the lower order veins (Fig. 2D). Altus and Canny (1985) and Nelson and Dengler (1997) reported that the conspicuous veins were running in parallel from the base to the apex of a leaf in grass leaves, which were interlinked by small higher order veins.

The mechanically stiff midribs in monocots are developed to tolerate mechanical stress along the longitudinal axis of the leaves (Givnish, 1979). The mechanical properties of grass leaves through characteristic parallel arrangement of veins were studied by Vincent (1982).

The parallel lower order veins develop high stability under unfavorable environmental conditions (Kull and Herbig, 1995). The architecture of the mineral and water transport network in monocots were extensively studied by numerous scientists (Canny, 1993; Altus *et al.*, 1985).

Trichomes were found to be absent in *G. superba*. The Calcium oxalate crystals were observed in highly specialized chambers on epidermis (Fig. 3A and 3B). Bulliform cells were also observed on the leaf margins (Fig. 3C and 3D).

The epicuticular wax deposition is of high systematic significance, which serves as a key for identification of major groups among monocotyledons. Con-vallaria type of wax deposition was observed in this plant species. The lamina consists of parallel oriented platelets, which occasionally formed striking patterns around idioblasts and stomata. Morphological and anatomical studies of Liliaceae members indicated the presence of parallel venation pattern and anomocytic stomata in *Lilium ledebouri* (Kaviani, 2008), *Tulip orphanidae* (Soykan and Meric, 2010), *Fritillaria caucasica* (Akyol *et al.*, 2014).

4. CONCLUSION

This is the first report on foliar micromorphological and architectural parameters in *G. superba*. This information could be useful in species identification, classification and establishing the taxonomic relationship with other family members and to understand the environmental influence on the physiology of plant. Due to scarcity of this species, adulteration in leaf drug of *G. superba* in the market samples could be identified through these parameters.

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