



# EPIDERMAL FEATURES, VENATION PATTERN AND PETIOLE ANATOMY OF *GARCINIA NIGROLINEATA* PLANCH. EX T. ANDERSON, NEWLY REPORTED SPECIES FROM NORTH EAST INDIA

Ajima Begum

Department of Botany, Tihu College, Tihu - 781371 (Assam), India.

## Abstract

Micromorphological and anatomical characters of leaves have been found as a taxonomic utility for delineation of closely related plant taxa. In the present study the foliar epidermal features, venation pattern and petiole anatomy has been used as a taxonomic tool to identify *Garcinia nigrolineata* Planch. ex T. Anderson, a newly reported species from North East India.

**Key words :** *Garcinia nigrolineata*, Micromorphology, Petiole anatomy, Stomata, Venation, Vein islet.

## Introduction

The genus *Garcinia* L. belongs to the family Clusiaceae (syn. Guttiferae) which contains about 35 genera and up to 800 species (Osman and Milan, 2006). The family is pantropical and comprises mostly large evergreen trees, or erect shrubs, with smooth, thin bark and white or yellow latex. The genus is native to South Asia starting from southern parts of the Thailand and Peninsular Malaysia to Indonesia but distributed throughout South East Asian region (Singh 1993; Mabberley 2005). Out of the estimated species worldwide, in India, Anderson (1874) reported 30 species in Flora of British India. Out of the 35 species reported by Maheswhari (1964), 15 species are from North-East India. Kanjilal *et al.*, (1934) reported 9 species from erstwhile Assam. However Begum *et al.*, (2014) added one more species of *Garcinia* i. e. *Garcinia nigrolineata* in the list of species of *Garcinia* of Assam. The species was collected from road side area of Gulai (N 27°22' 13.69", E 95°37'19.22", Alt. 101m), Digboi, Tinsukia district of Assam and is a new record for its distribution in Assam as well as in North East India. However Scrutiny of literatures reveals that the above species has not been reported earlier from India.

The present study is carried out to explore the foliar epidermal features, venation pattern and petiole anatomy of *Garcinia nigrolineata*. Aworinde *et al.*, (2009) considered that the data generated from leaf epidermal studies in solving taxonomic problem in plants have gained much recognition for a very long time. They revealed that the epidermal and cuticular features of plants could serve as a vital tool usable in the systematic of present day angiosperm. Yasmin *et al.*, (2009) revealed that foliar epidermal feature particularly the stomata have proved very useful in delineation of doubtful families. Hickey (1973) stated that leaf venation is correlated with plant evolution and has systematic significance in plant identification and classification. Indeed, leaf venation plays a very important role in the identification of incomplete plants, e.g., sterile specimens, archaeological remains and fragmentary fossils of non-reproductive organs. Howard (1962) considered that the vascular bundle of the petiole is significant in the identification of vascular plant species. Metcalfe and Chalk (1950) and Schofield (1968) emphasized that the vascular pattern of the petiole is very helpful in identification of species within Clusiaceae and they identified six distinct types of vascular structures. It has been found that the morphological studies along with anatomical features are important for solving taxonomic and evolutionary issues as well as establishing

\*Author for correspondence : E-mail: azimabio@gmail.com

the relationships among *Garcinia* species (Pathirana and Herat 2004). The major objective of the present work is to present a precise characterization of foliar epidermal features, venation pattern and petiole anatomy so that the identification of this species will be more confirmed along with morphological attributes.

#### Taxonomy of *G. nigrolineata*

Trees 14 m tall, 45 cm in diam. Bark blackish grey, smooth but in some portions cracks has been found. Wood light pink exuding yellow latex frequently. Leaves 10 - 19 cm x 2.8 - 6.3 cm, shiny, dark green above and light green underneath, coriaceous, oblong lanceolate with acuminate, rarely acute or obtuse apex and attenuate base. Male flower 1.27 cm wide, on stalks 1 cm- 1.5 cm long. Female flowers 1.27 cm – 1.5 cm wide; the petals rather long and narrow, yellow. Fruit 5 - 8 cm x 4 - 6 cm, sub-globose, beaked or pointed, faintly ribbed, yellow orange when ripe (Plate No. 1; Fig. a-i).

#### Materials and Methods

Both the fresh and preserved leaf samples in 4% Formaldehyde were used to study the foliar epidermal features, venation pattern and anatomical features of petiole. The protocol suggested by Radford *et al.*, (1974) was used with slight modification for separating epidermal layer from the leaf blade. The leaves were placed in fresh 5% NaOH (Sodium hydroxide) solution, which was replaced at the interval of two days for 10 - 15 days. Leaves were then washed properly with water and dipped in 3% H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide) solution for 12 hours. When the epidermal layers got completely separate from the rest of the tissues, the separated layer was then taken out with the help of forceps. The epidermal layer was then stained with 1% safranin and mounted in glycerine and sealed with DPX. To study leaf architecture, the same method given by Radford *et al.*, (1974) for foliar epidermal study with slight modification was adopted.

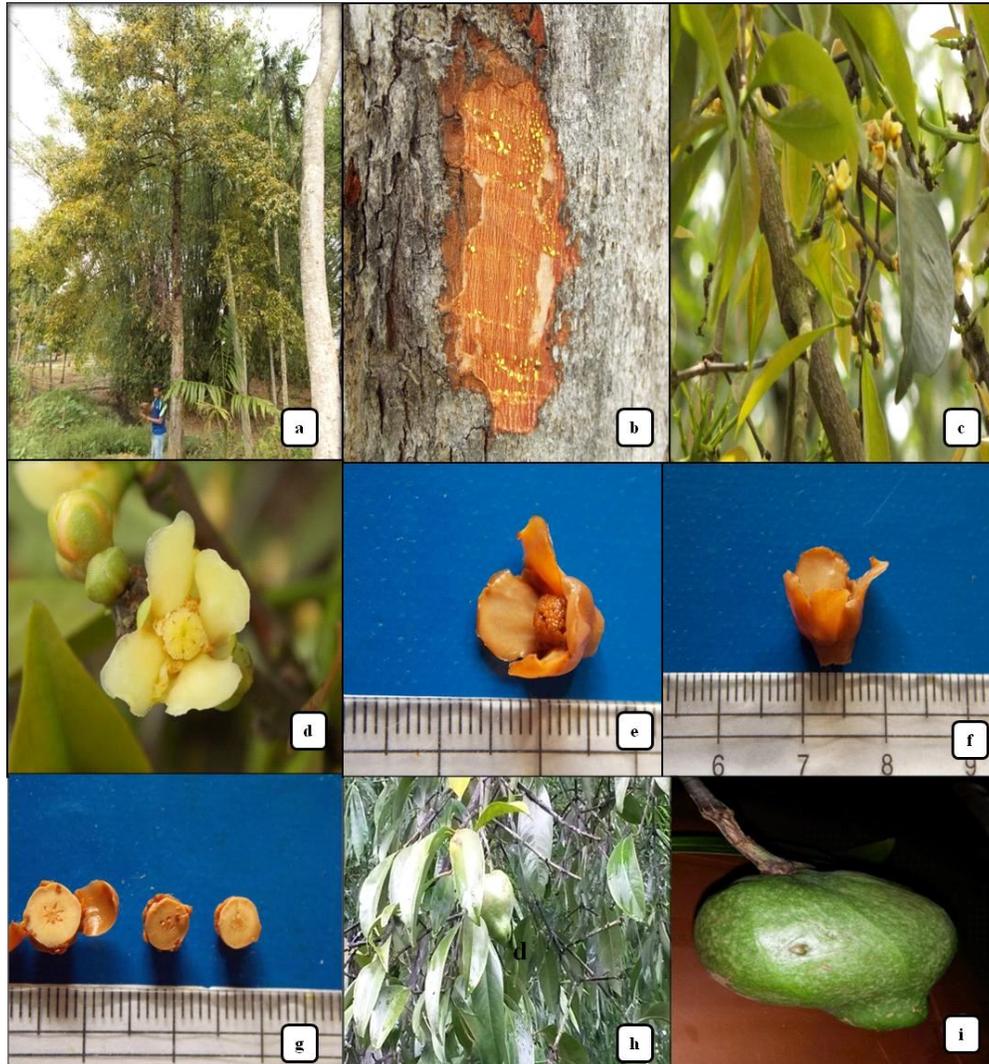


Plate No. 1: *G. nigrolineata*: Fig. a-i. a. Habit; b. Blaze; c & d. Twig of flowers; e. & f. Flower in the scale of length and breadth resp.; g. T.S. of ovary; h & i. Fruit.

Here after treatment with Hydrogen peroxide for 12 hours, the leaves were washed with water and stained with safranin. The leaves were then taken on glass petridishes and observed under microscope in different magnification ranges from 10X to 100X.

Microphotographs of leaf venation of each taxon were taken with the help of Stereozoom Microscope (Leica). In describing venation pattern, the terminology suggested by Hickey (1973), Melville (1976) and LAWG (1999) was followed.

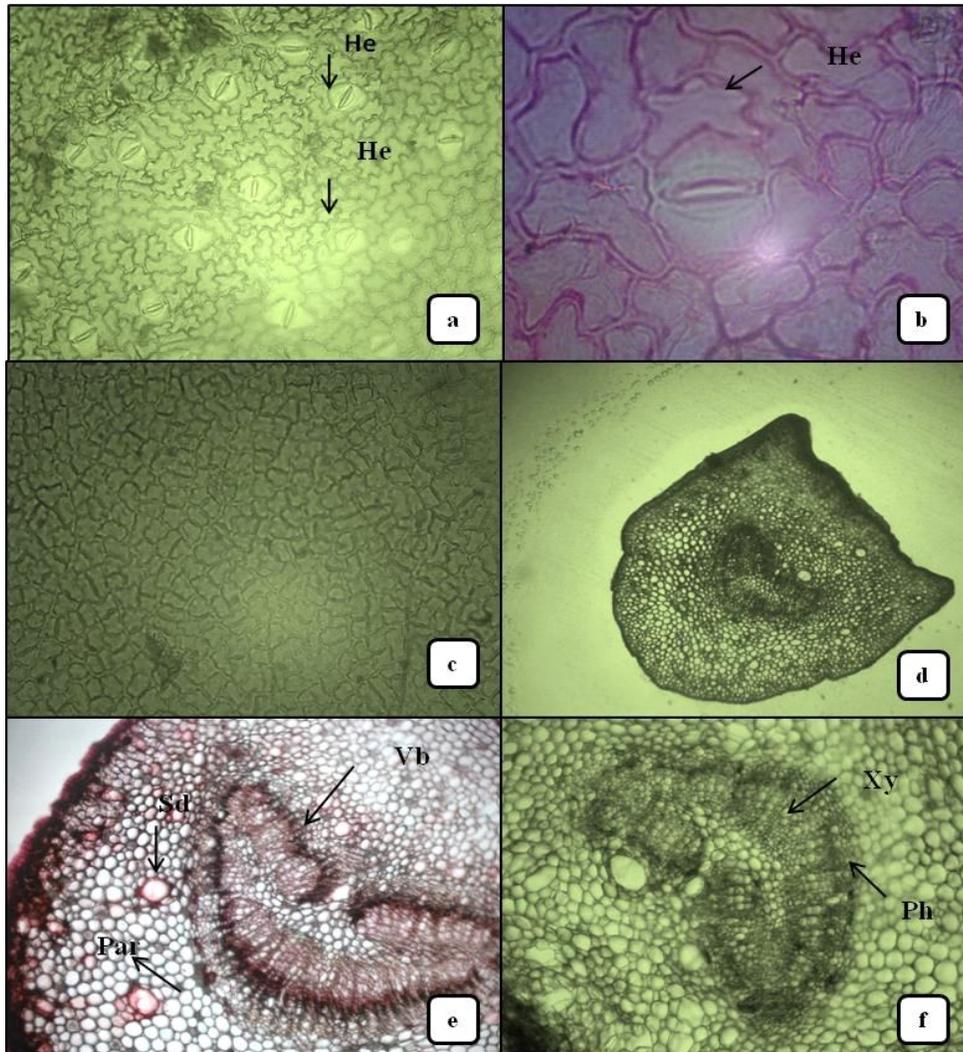
To make the slide of petiole, thin sections were made and permanent slides were prepared by following the methods suggested by Radford *et al.*, (1974) and Gerlach (1977). The sections were stained with safranin and fast green and subsequently dehydrated through an ethanol

gradient (70% to 100%). The sections were then treated with xylol and mounted on slides using DPX. The slides were then studied under compound microscope (Leica) using 40X and 100X magnifications and photographs were taken.

## Results and Discussion

### Foliar epidermis

Leaves hypostomatic, the stomata are confined to the abaxial surface only; the stomata are irregularly distributed and the axes of stomata are randomly oriented; the most common arrangement of stomata are hemiparacytic mixed with few anomocytic; the stomata are surrounded by 1 - 5 subsidiary cells, very few stomata are surrounded by 8 subsidiary cell forming rosette form;



**Plate No. 2: *G. nigrolineata* : Fig. a-f.** a & b. Stomata of lower surface in 100X and 400X resp.; c. Upper epidermal cell. **Fig. d- f. T.S. of petiole** . d. Outline of petiole in 40X.; e & f. T. S. of petiole in 100X. **Abbreviations:** He.- Hemiparacytic; Par- Parenchyma; Vb- Vascular Bundle; Xy-Xylem; Ph-Phloem; Sd- Secretory duct

stomatal size:  $25.8 \mu \times 36 \mu$ ; stomatal frequency:  $90.6 \text{ mm}^2$  and stomatal index: 20.4. The intercostal epidermal cells are irregular with highly sinuous anticlinal wall and coastal cells are trigonal to tetragonal with sinuous anticlinal wall, 9 - 10 layered in both the surfaces. Contiguous stomata present (Plate No. 2; Fig. a-c).

### Venation pattern

Mid vein raised abaxially. Venation is weak brochidodromous. Primary vein is stout, straight. Secondary veins are sub opposite, curved abruptly, 12 - 15 on each side of the lamina with 0.6 - 1.5 cm space in between them; angle of divergence is wide to narrow acute *i.e.* lower secondary veins are  $65^\circ - 80^\circ$  wide and upper veins are  $40^\circ$  wide. Composite intersecondary veins

arises from  $1^0$  and  $2^0$  veins. Tertiary veins are orthogonal reticulate arises both from  $1^0$ ,  $2^0$  and intersecondary veins; the angle of origin with respect to  $2^0$  is AO or AR. Higher vein order have been resolved upto  $6^{\text{th}}$ . The areoles are well developed, trigonal to polygonal with random arrangement. Veinlets entering in the areoles are curved, branched irregularly with 1 - 8 free vein endings. The tracheidal ends are elongated. Marginal ultimate venation is incomplete (Plate No. 3; Fig. a-h).

### Petiole anatomy

The outline of petiole is blunt with two lateral outgrowths in adaxial surface and convex in abaxial surface. Epidermis is single layered; cortex is made up of 5 layered angular collenchymas cells and 10 - 11

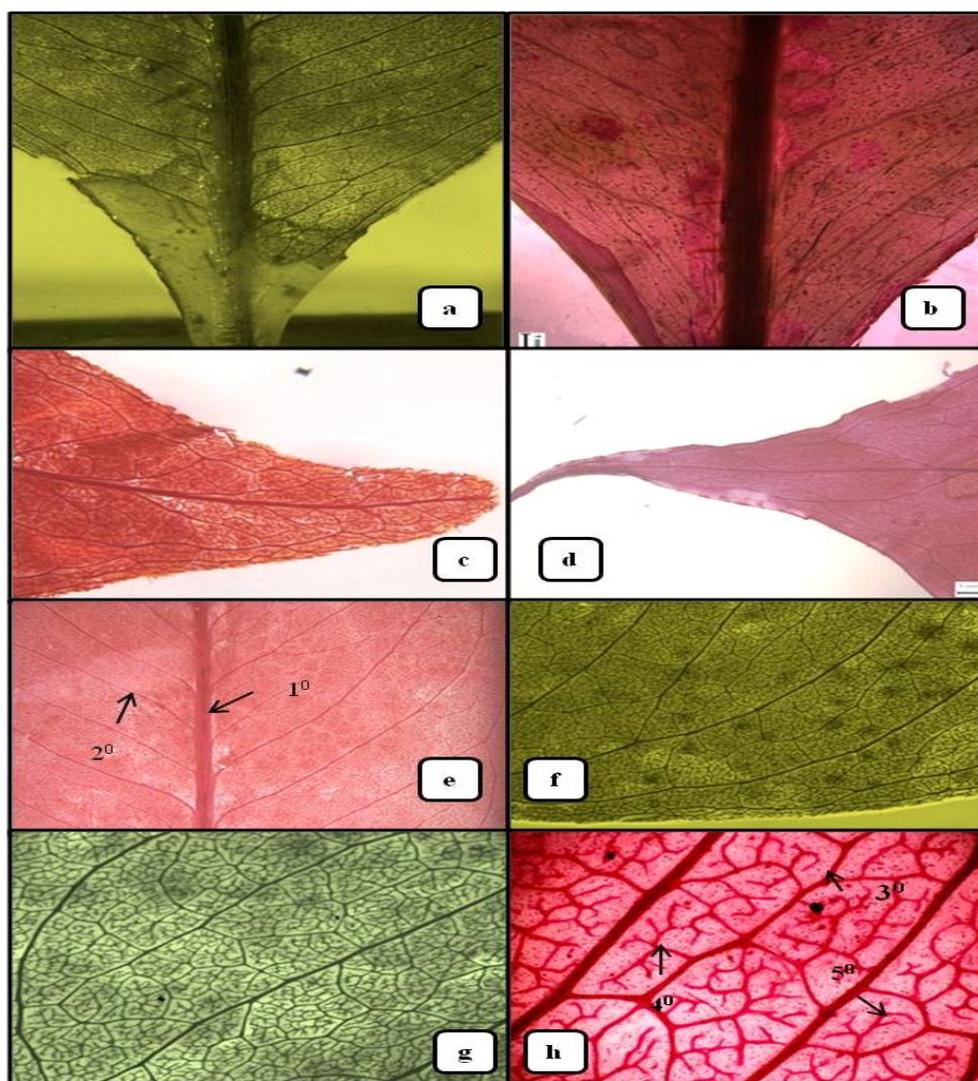


Plate No. 3: *G. nigrolineata*. Fig. a- h. Venation pattern. a & b. Base 10X; c & d. Apex 10X; e, g & h. Middle portion 10X, 20X, 40X resp.; f. Margin 10X. Abbreviations:  $1^0$  - Primary vein;  $2^0$  - Secondary vein;  $3^0$  - Tertiary vein;  $4^0$  - Quarter-nary vein;  $5^0$  - Higherorder vein.

layered thin walled spherical parenchyma cells. Vascular bundle is arc shaped with incurved distal ends which are not fused towards the adaxial side. The vascular bundle is continuous, unbroken and surrounded by 7 layered thick bundle sheath. The secretory canals handsomely surround the vascular bundle from all sides. The secretory canals found in the abaxial side of the bundle are large in comparison to that of adaxial side (Plate No. 2; Fig. d-f).

### Conclusion

The foliar micromorphological, leaf architectural parameters and petiole anatomy of *Garcinia nigrolineata* could be significant in identifying the species along with its morphological attributes since most of the species of *Garcinia* are very much alike which often creates hindrance in its identification. Moreover these features could be applied in studying the environmental impact on the structure, orientation and density of leaf constants such as stomatal index, frequency and vein density.

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