Indian J. Applied & Pure Bio. Vol. 39(1), 585-594 (2024).

ISSN: 0970-2091
A Web of Science Journal

Morphological study and analysis of leaf architecture in two primary and two secondary food plants of muga silkworm, *Antheraea assamensis* Helfer in Assam

[1]Abdul Jalil, [2]Bedabati Chowdhury and [3]Azizur Rahman

[1]Department of Botany, Hatsingimari College, Hatsingimari - 783135 (India)
[2]Department of Botany, University of Science and Technology, Meghalaya - 793101 (India)
[3]Department of Sericulture, Government of Assam, Guwahati - 781022 (India)
Email: abduljalil4all@gmail.com

Abstract

Muga silk production is a pride of Assam. About 6% of the natural silk produced in the world is bagged by muga (*Antheraea assamensis* Helfer). The muga rearing is very much dependent on its food plants which are mainly perennial tree available in the vast geographical region of North East India. While *Persea bombycina* Kost (Som) and *Litsea monopetala* Roax (Soalu) (Family: Lauraceae) are predominantly used as the primary food plants for rearing of muga silkworm and *Litsea salicifolia* Hook. and *Litsea cubeba* Lour. are used as secondary food plants for rearing of muga silkworm (*Antheraea assamensis* Helfer). The leaf architecture in these food plants are diversed. The objective of the study is to reveal the morphological study and analysis of leaf architecture of those four primary and secondary food plants of muga (*Antheraea assamensis* Helfer). The results of leaf micro morphology studies like, lamina shape, venation framework, stomata, trichomes etc can provide more accurate basis for the selection of food plants for the rearing purposes of muga silkworm.

Key words : *Persea, Litsea,* leaf architecture, muga silkworm, *Antheraea assamensis* Helfer.

Sericulture plays a pivotal role in the rural economy of Assam. The climatic and geographical status of Assam has made sericulture one of the major economical livelihoods in this region. Muga silk industry has been playing an important role in uplifting the socioeconomic conditions of the people of this region and improving the rural economy. The accomplishment in sericulture industry not only depends on appropriate environmental condition but proper selection of food plants also plays an important role in its success. The leaf protein would be ultimately converted into silk protein and therefore the economic characters of cocoons, the growth and development of larvae is largely influenced by the nutritional quality of leaves fed by. Since the leaf quality has significant effect on the quality and quantity of silk fiber. The morphological study and leaf architecture analysis of (1) primary food plants: *Persea bombycina* Kost and *Litsea monopetala* Roxb., and (2) secondary food plants: *Litsea salicifolia* Hook and *Litsea cubeba* Lour were done with an aim to correlate the data to the quality of silk fibers.

These food plants are belonging to the laurel family Lauraceae (order: laurales). Kostermans[21] had subdivided lauraceae into two subfamilies, namely Lauroideae and Cassythoideae. The family comprises about 2850 known species in about 45 genera worldwide[5]. This family includes mostly evergreen shrubs and trees which are distributed throughout tropical and subtropical regions; principally Southeast Asia and tropical America, particularly Brazil. The family contains a number of economically important trees, including Som, soalu, avocado, cinnamon, bay and a variety of valuable timber trees, the wood of which sometimes remains fragrant for decades after it is cut.

Persea consists of about 150 species of evergreen trees and among these *Persia bombycina* Kost. is the primary food plants of muga silkworm *Antheraea assamensis* Helfer. The another genus Litsea is estimated that there are about 300 species of it distributed in tropical Asia and in islands of Australia, New Zealand, North and Central America[31]. In India about 45 species are distributed in evergreen and semi evergreen forests of peninsular India, 40 of which are endemic to peninsular India[2]. Among which 12 species are found in Karnataka[34] and some species are also found in Meghalaya, Manipur, Assam and Sikkim states.

There are many disciplines including leaf morphology associated with plant taxonomy, which are used by taxonomists as an aid, or to improve the identification, classification and systematic position of plant taxa. Among these disciplines leaf architecture is one of the most significant tools used by taxonomist to identify and differentiate closely related taxa. For ecology, systematic study of plant, conservation and paleobotany, the leaf architechture plays a key role[11].

Generally the stomata are found only on the lower surface of leaf of most of the tree species; amphistomatous leaves are such leaves which have on both upper and lower leaf surface stomata; hypostomatous leaves are such leaves which have only on lower leaf surface stomata, and epistomatous or hyperstomatous leaves are such leaves which have only on upper leaf surface stomata[37].

Dilchar[10] classified the stomata based on the arrangement of subsidiary cells in the mature stomatal complex of vasculasr plants. The guard cells of most of the tree species are generally kidney shaped and in generally the guard cells found in grasses are dumbbell shaped[13].

The stomata can absolutely affect on global carbon and water cycles as well as play a key ability important for plants grow and survive abiotic and biotic stress, plants compete for resources including nutrients, light, water, space etc particularly under non-optimal conditions of crop products that are likely complicated by the present days climate change mainly the increasing atmospheric Carbon dioxide[15,30].

The vein orders have vast diversity in the angiosperm plants which provide great architectural elements, which is a hierarchy of vein systems forming a reticulate mesh[11,15,23].

For plant identification and plant taxonomy; the type, location, presence and absence of trichomes on leaf surface are effectible diagnostic features in plant identification and plant taxonomy[7]. To avoid abiotic stressors, including water losses, extreme temperatures and UV radiation, and biotic threats, such as pathogen or herbivore attack; non-glandular trichomes support a structural protection[19].

The healthy and mature leaves of the primary (*Persea bombycina* Kost. *& Litsea monopetala* Roxb.*)* and secondary *(Litsea salicifolia* Hook. and *Litsea cubeba* Lour.) host plant species of muga silkworm *Antheraea assamensis* Helfer were collected from the study area, Regional Muga Research Station (RMRS), Boko, Kamrup, Assam and Directorate of sericulture, Khanapara, Assam from January 2019 to April 2021. For the analysis of leaf architechture the parameters such as chief leaf type, phyllotaxy, kind of leaf, shape of leaf, petiolate, venation pattern, types of stomata, types of trichomes, margin of leaf, of the leaf were studied[8]. Analysis was performed under stereomicroscope.

Since the leaves are densely pubescent, for foliar venation studies the leaves were put under dissecting microscope and after brushing off the hairs with the help of soft dental brush as much as possible before clearing the vein, the leaf veins were studied[1,35] except *Litsea cubaba* Lour. which is lack of trichomes.

Venation patterns were investigated following a modified protocol of Payne[31]. One to several leaves of the primary (*Persea bombycina* Kost. & *Litsea monopetala* Roxb.) and secondary (*Litsea salicifolia* Hook. and *Litsea cubeba* Lour.) host plant species were collected into petri dishes and immersed in 10% NaOH at room temperature until they became translucent. This step took one to three weeks, depending on species; thick, leathery or stone-like leaves took longer. Then the leaves were washed twice with water and bleached in 50% commercial bleach for 10 minutes. After another two washing processes, the leaves were dehydrated for 10 minutes successively in 50% and then 75% ethanol. Leaves were then stained by 1% Safranin in 95% ethanol for 20 minutes. Stained leaves were washed for a minute or two, depending on leaf thickness, with absolute ethanol to de-stain non vascular tissues. Preparations were then immersed in absolute ethanol: Histo-Clear (1:1) mixture, and then removed with fine forceps to be mounted in DPX resin between glass slides and cover slips for permanent preservation.

The following vein characters were analyzed: 1) Primary Vein Framework; 2) Major Secondary Vein Framework; 3) Perimarginal veins; 4) Intercoastal Tertiary Vein Fabric; 5) Quaternary Vein Fabric; and 6) Freely Ending Vein let (FEVs). For the FEVs, where more than one type was detected, we categorized it according to the most common type[1].

Table-1. Leaf morphology and architecture characters

Leaf characters/ Species	*Persea bombycina* Kost	*Litsea monopetala* Roxb.	*Litsea salicifolia* Hook.	*Litsea cubeba* Lour.
Lamina Shape	Elliptic-lanceolate to obovate-lanceolate, oblong-lanceolate or oblanceolate	Oval to obovate,	Narrow-lanceolate or elliptic-oblong, acute or acuminate	Lanceolate or narrow ovate-lanceolate, caudate, acuminate, membranous
Apex	Apex acuminate or sub-acuminate; Cuneate or acute	Apex convex	Acute	Acute
Base Above Beneath	Coriaceous, glabrous. Adpressed silky beneath when young, minutely silky or puberulous with age	Rounded, somewhat oblique Coriaceous, glabrescent Glaucous and rusty-pubescent	Cuneate Dark green above Chartaceous, glaucous and minutely silky pubescent beneath	Cuneate Bright green above, Glaucous beneath
Venation framework	The primary veins (1°) pattern pinnate	The primary veins (1°) pattern pinnate	The primary veins (1°) pattern pinnate	The primary veins (1°) pattern pinnate
2° vein category	Reticulodromous	Weak brochidodromous	Reticulodromous	Reticulodromous
2° vein spacing	Irregular	Uniform	Increasing toward the base	Irregular
2° vein angle	Slightly increasing toward the apex	Uniform	Uniform	One pair acute basal secondary
Inter 2° vein	Less numbered and comparatively very thin	Absent	Absent intersecondaries	Weak
3° vein	Mixed opposite percurrent/alternate percurrent	Mixed opposite percurrent/ alternate percurrent	Mixed opposite percurrent/alternate percurrent	Regular polygonal reticulate, inconsistent,
3° vein course	Sinous	Straight and sinous	Sinous	Exmedially ramified
3° vein angle to 1°	Acute	Obtuse	Acute	Obtuse
4° vein	Alternate percurrent	Alternate percurrent	Regular polygonal reticulate	Regular polygonal reticulate

5° vein	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate
Areolation	Well developed areolation	Moderately developed areolation	Well developed areolation	Paxillate areolation
FEVs	Branched	1-branched	Unbranched	1-branched
Types of stomata	Anomocytic	Anomocytic	Anomocytic and Paracytic type	Paracytic and Amphiparacytic type
Trichomes	Unbranched, unicellular	Unbranched, unicellular	Unbranched, unicellular	Absent

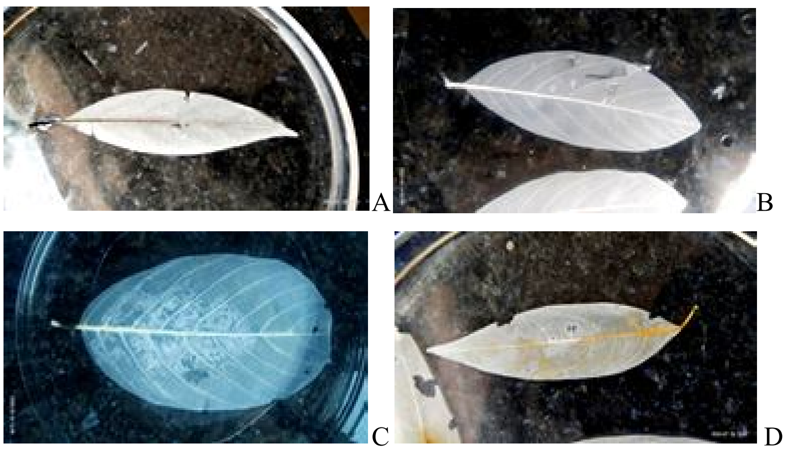

Fig 1: Leaf shape and leaf vein of

A: *Persea bombycina* Kost.

B: *Litsea salicifolia* Hook.

C: *Litsea monopetala* Roxb.

D: *Litsea cubeba* Lour.

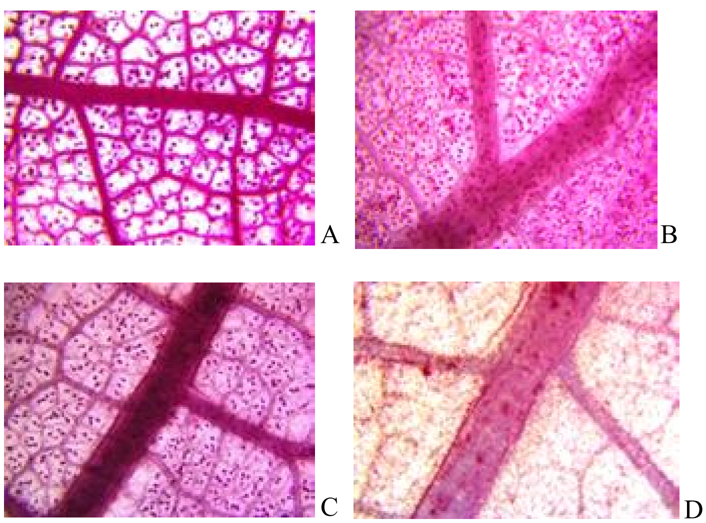

Fig 2: Leaf middle portion and vein pattern

A: *Persea bombycina* Kost.

B: *Litsea salicifolia* Hook.

C: *Litsea monopetala* Roxb.

D: *Litsea cubeba* Lour.

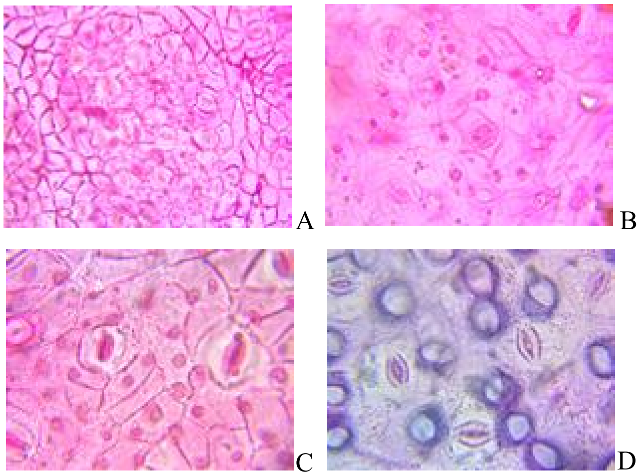

Fig 3: Type of stomata in
A: *Persea bombycina* Kost. (Anomocytic type)
B: *Litsea salicifolia* Hook. (Anomocytic and Paracytic type)
C: *Litsea monopetala* Roxb. (Anomocytic type)
D: *Litsea cubeba* Lour. (Paracytic and amphiparacytic type)

Fig 4: Types of Trichomes in
A: *Persea bombycina* Kost. B: *Litsea salicifolia* Hook. C: *Litsea monopetala* Roxb.

To study the stomata structure, the segments or leaf pieces were boiled in concentrated HNO_3 and also a small amount of $KClO_3$ was added. The pieces become brown and gradually yellowish white. These were carried to water which help to get the leaf peelings. Peels were clean in water, and then stained with the help of aqueous Safranin and then mounted in Glycerin for observation[4]. The standard terminology to describe the stomata of the food plants is used in accordance with Dichler[10].

For trichome study under light microscope, using the leaf sample from formalin: acetic acid: alcohol (FAA) and also preserved sample was kept in glvcol methacrylate (GMA) following to the modified mathod of Feder & O'Brien[14] and several leaf sample of each food plant were examined under a stereomicroscope.

Persea bombycina Kost :

Leaves petiolate, alternate, simple, lamina shape elliptic-lanceolate to obovate-lanceolate, oblong-lanceolate or oblanceolate; apex acuminate or sub-acuminate; coriaceous, glabrous above, adpressed silky beneath when young, minutely silky or puberulous with age; base cuneate or acute. These features of leaf texture changes from morphotypes to morphotypes. Tazima and Choudhury (2005) reported five ecotypes of Som based on the shape of the leaves: ‘‘Naharpatiya,’’ ‘‘Azarpatiya,’’ ‘‘Ampatiya,’’ ‘‘Jampatiya,’’ and ‘‘Bahpatiya.’’ As leaves are the solitary dietary source for the muga silkworm, which converts them directly to silk fibers, high-value silk production requires improved leaf quality of the host plant. Researchers have reported variations among Som (*Persia bombycina* Kost.) genotypes based on morphology and taste of leaves.

The primary veins pattern pinnate, 2° weak brochidodromous, 2° vein spacing irregular, 2° vein angle slightly increasing toward the apex , intersecondary veins less numbered and comparatively very thin, 3° vein mixed opposite percurrent/alternate percurrent, sinous, acute, alternate percurrent 4° vein, 5° vein regular polygonal reticulate, well developed areoles, 1 branched FEVs, thin highest excurrent, looped with no teeth. Unicellular, unbranched, trichomes are more abundant on the abaxial surface than the adaxial surface of the leaves.

Upper epidermis: - Stomata are absent on upper epidermis. The shapes of epidermal cells of this species are near about polygonal and irregular.

Lower epidermis: - Stomata are anomocytic type. The kidney shaped guard cells of this species are elongated. Epidermal cells of this species are polygonal shape and irregular.

Litsea monopetala Roxb.*:*

Leaves petiolate, alternate, simple, lamina shape oval to obovate, apex convex coriaceous, glabrescent above, glaucous and rusty-pubescent beneath; base rounded, somewhat oblique; The primary veins (1°) pattern pinnate, 2° weak brochidodromous, 2° vein spacing near about uniform, 2° vein angle uniform, intersecondary veins absent, 3° vein mixed opposite percurrent/alternate percurrent, straight and sinous mix, obtuse, alternate percurrent 4° vein, 5° vein regular polygonal reticulate, moderately developed areoles, 1 branched FEVs, highest excurrent, looped with no teeth.

Unicellular, unbranched, trichomes are more abundant on the abaxial surface than the adaxial surface of the leaves.

Upper epidermis: - Stomata are absent on upper epidermis. The shapes of epidermal cells of this species are near about polygonal and irregular.

Lower epidermis: - Stomata are anomocytic type. The kidney shaped guard cells of this species are elongated. Epidermal cells of this species are polygonal shape and irregular.

Litsea salicifolia Hook.

Leaves petiolate, alternate, simple, lamina shape narrow-lanceolate or elliptic-oblong, acute or acuminate, dark green above, chartaceous, glaucous and minutely silky pubescent beneath.

The primary veins (1°) pattern pinnate, 2° vein spacing increasing toward the base, 2° vein angle uniform, 2° intersecondaries absent, 3° vein mixed opposite percurrent/ alternate percurrent, sinuous, acute, thin 4° & 5° vein regular polygonal reticulate, well developed areolation, unbranched FEVs, highest excurrent, looped with no teeth. Unicellular, unbranched, trichomes are more abundant on the abaxial surface than the adaxial surface of the leaves.

Upper epidermis: - Stomata are absent on upper epidermis. The shapes of epidermal cells of this species are near about polygonal and irregular.

Lower epidermis: - Stomata are Anomocytic and Paracytic type. The kidney shaped guard cells of this species are elongated. Epidermal cells of this species are polygonal shape and irregular.

Litsea cubeba Lour :

Leaves petiolate, alternate, simple, lamina shape lanceolate or narrow ovate-lanceolate, caudate- acuminate, membranous, bright green above, glaucous beneath.

The primary veins (1°) pattern pinnate, 2° vein spacing irregular, 2° vein angle one pair acute basal secondaries, weak intersecondaries, 3° vein regular polygonal reticulate, inconsistent, exmedially ramified, obtuse, 4° vein regular polygonal reticulate, 5° vein regular polygonal reticulate, paxillate areolation, 1-branched FEVs, highest excurrent, looped with no teeth.

Trichomes are absent in the mature leaves.

Upper epidermis: - Stomata are absent on upper epidermis. The shapes of epidermal cells of this species are near about polygonal and irregular.

Lower epidermis: - Stomata are Paracytic (Rubiaceous type) and Amphiparacytic type. The kidney shaped guard cells of this species are elongated. Epidermal cells of this species are polygonal shape and irregular.

This work on leaf micro morphology or leaf architecture pattern, is stable and can be help full to use as the more beneficial food plants with success in different rearing seasons. This study also shows the taxonomic importance without the using of reproductive body part which has the practical and theoretical relevance and significance. This study will bear the ability to help to expand leaf micro morphological findings of different food plants for rearing purposes of silkworm.

References :

1. Antar *et al*., (2022), *Acta Botanica Brasilica, 36:* doi: 10.1590/0102-33062021abb0183.
2. Bhuniya T, P. Singh and SK Mukherjee (2010), *Journal of Plant Taxon 17:* 183-191.
3. Singh Bhau Brijmohan, (2009), *Biochem Genet 47:* 486–497, DOI 10.1007/ s10528-009-9242-6.
4. Chachad D. P. (2016), *World Journal of Pharmaceutical Research, 5*(3): 1060-1068.
5. Christenhusz, Maarten J.M. and James

W. Byng, (2016). *Phytotaxa. 261* (3): 201–217. doi:10.11646/phytotaxa.261.3.1.

6. Dale, M. B., R. H., Groves, V. J Hull, and J.F O'Callaghan, (1971). *New Phytologist 70:* 437-442.
7. Davis, P. H. and V.H. Heywood, (1963). Principles of angiosperm taxonomy, p. 154, Princeton, New Jersey: Van Nostrandpage.
8. Dhar T. Praveen., (2017), *International Journal of Advanced Research in Biological Sciences. 4*(5): 182-185.
9. Dickinson, T.A., W.H Parker, R. E Strauss (1987), *Taxon 36:* 1-20.
10. Dilcher, D.L. (1974) *Botanical Review 40 :* 1-157.
11. Ellis, B., D. C. L. J Daly,. J. D Hickey, K. R Mitchell,. P Johnson, Wilf and S.L. Wing (2009), Manual of Leaf Architecture, 190 pp, Cornell University Press. New York, USA.
12. Ettingshausen, C. (1861). *Die Blatt-Skelete der Dicotyledonen. Vienna 1:* 21.
13. Evert, R.F. (2006). Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. Hoboken, NJ: Wiley Interscience.
14. Feder. N. and O Brien. T.P (1968). *American Journal of Botany 55:* 123-142.
15. Hetherington AM and FI Woodward (2003) *Nature 424:* 901–908
16. Hickey, Leo J. (1973), *American Journal of Botany 60:* 17-33.
17. Hickey, Leo J. (1977), Geological Soceity of America Memoir 150.
18. Jensen, R. J. (1990), *American Journal of Botany 77 :* 1279-1293.
19. Kanjilal and Bor, (1997), Flora of Assam, Fam 115 Lauraceae, Omsons Publications, New Delhi, 66-87.
20. Karabourniotis, G., G Liakopoulos, D Nikolopoulos and P Bresta (2020). *Journal of Forestry Research. 31* (1): 1–12. doi:10.1007/s11676-019-01034-4.
21. Kostermans, (1957), *Reinwardtia, Herbarium -Bogoriense, Kebun Raya Indonesia, 4*(2): 193–256.
22. Manchester, S. R. (1986), *Botanical Gazette 147:* 200-226.
23. McKown AD, H Cochard, and L Sack, (2010). *American Naturalist 175:* 447–460.
24. Melville, R. (1937), *Annals of Botany, 1:* 673-679.
25. Melville, R. (1976). The terminology of leaf architecture. *Taxon 25:* 549-561.
26. Merrill, E. K. (1978). *Botanical Gazette 139:* 447-453.
27. Mondal, A.K. (2005), Advanced plant taxonomy, Taxonomy- A synthetic subject, pp. 243-261., New central book agency(P) Ltd, Kolkata, India.
28. Mouton, J. A. (1966). *Bulletin de la Société Botanique de France 113:* 492-502.
29. Mouton, J. A. (1967). *Congres national des sociétés savantes 92:* 165-176.
30. Myers SS, A Zanobetti and Kloog I, (2014), *Nature 510:* 139–142.
31. Ngernsaengsaruay C, DJ Middleton and K Chayamarit (2011), *Thai Forest. Bulletin. (Botany.) 39:* 40–119.
32. Rabiae *et al*., (2020), *International Journal of Pharmacy & Life Sciences, 11*(1): 6478-6481.

33. Ray, T. S. (1992), *American Journal of Botany 79:* 69-76.
34. Saldanha CJ (1996) Flora of Karnataka. Oxford and IBH publishing Ltd, Vol 1, New Delhi .
35. Vasco *et al*., (2014), *Applications in Plant Sciences 2*(9): 1400038, doi:10.3732/apps.1400038.
36. Webb, L.J. (1955), J*ournal of Ecology 47:* 551-570.
37. Willmer, C and M. Fricker (1996). Stomata. Springer. p.16. doi:10.1007/978-94-011-0579-8.
38. Wolfe, J. A. (1978), *American Scientist 66:* 694-703.
39. Wolfe, J.A. (1993). A Method of Obtaining Climatic Parameters from Leaf Assemblages. USGS Bulletin, 2040.