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**Satheesh K,**

Centre for Medicinal Plants Research,  
Arya Vaidya Sala, Kottakkal,  
Changuvetty, Malappuram Dist. –  
676 503.

**Remashree\*A.B,**

Centre for Medicinal Plants Research,  
Arya Vaidya Sala, Kottakkal,  
Changuvetty, Malappuram Dist. –  
676 503.

**Anilkumar N.1,**

M.S. Swaminathan Research  
Foundation, Taramani Institutional  
Area, Chennai-600 113

**Indira Balachandran,**

Centre for Medicinal Plants Research,  
Arya Vaidya Sala, Kottakkal,  
Changuvetty, Malappuram Dist. –  
676 503.

**Correspondence:****Remashree A.B,**

Centre for Medicinal Plants  
Research, Arya Vaidya Sala,  
Kottakkal, Changuvetty,  
Malappuram Dist. – 676 503.  
E-Mail: [remashree@gmail.com](mailto:remashree@gmail.com)

## Micromorphological Studies Using Scanning Electron Microscope (Sem) For Species Delimitation in The Selected Species of *Gymnema* Occurring in Kerala

Satheesh K, Remashree\*A.B, Anilkumar N & Indira Balachandran

### ABSTRACT

Micromorphological studies using SEM were helped in comparing four species of the genus *Gymnema* viz. *Gymnema sylvestre* (Retz.) Schult, *Gymnema mohanramii* Karthik & Moorthy, *Gymnema elegans* Wight & Arn., *Gymnema latifolium* Wall. ex Wight and formulation of new key method for identification.

**Keywords:** *Gymnema*, Micromorphology, SEM studies.

### 1. Introduction

The selected species of *Gymnema*, viz. *G. elegans*, *G. mohanramii*, *G. sylvestre* and *G. latifolium* are selected for the study. Except *G. sylvestre*, all are endemic. *G. latifolium* is very rare and endemic to the Western Ghats of Maharashtra and Kerala. This endemic species has been assigned the status of endangered, endemic red listed medicinal plant. *G. elegans* is endemic to Peninsular India and is rare. *G. mohanramii* is reported only from India. *G. sylvestre* is widely distributed and used against diabetes [1]. The present study is to delimit the existing confusion in the systematics of the genus *Gymnema*.

### 2. Materials and Methods

Leaf samples were collected and authenticated. Herbarium sheets were preserved at the Centre for medicinal plants Research, Arya Vaidya Sala, Kottakkal. Leaves were cut into a size of 1 cm<sup>2</sup>. Samples were processed for micro morphological, histological and Scanning Electron Microscopic studies [2]. Photographs were taken using Carl Zeiss compound microscope and canon G4 camera and Hitachi scanning electron microscope.

### 3. Results & Discussion

T.S of Leaf is dorsiventral and shows striated cuticle, followed by single layered epidermis. The uniseriate and multicellular trichomes are observed on the lower and upper epidermis. The upper epidermal cells are hexagonal while the lower epidermal cells are slightly wavy in surface view. Parasitic stomata are seen on the surface view. The single layered closely arranged palisade cells are present just below the upper epidermis. The spongy parenchyma is 3 to 5 cells thick with large intercellular space. The midrib region has amphicribal vascular bundle; lactifers and crystals of calcium oxalates are present in the parenchyma tissue around the vascular bundle. The histological similarities and variations are observed between species (Table .1). Well defined anatomical markers were found out and made a key to delimit the 4 species of *Gymnema*.

**Table 1:** The comparative histological details of Genus *Gymnema*

| Characters                   | Plant Species   |  |  |   |
|------------------------------|---|--|--|---|
|                              | <i>G. sylvestre</i>   | <i>G. elegans</i>  | <i>G. mohanramii</i>   | <i>G. latifolium</i>  |
| <b>Trichomes</b>             | Very long (250µm - 450µm)<br>Present on midrib<br><br>Width (10µm -15µm),<br><br>Uniseriate and multicellular | Moderate length (200µm - 300µm)<br>Present on midrib region and few are present on lower epidermis of the lamina<br>Width (10µm -15µm),<br>Thick walled uniseriate and multicellular | Medium sized length ranges from (50µm - 200µm)<br>Present on midrib and lamina<br><br>Width (18µm -25µm),<br><br>Thickwalled uniseriate and multicellular (2-5 cells)<br><br>Trichomes are suberised | Very long and stout (150µm - 400µm)<br>Present on midrib and lamina<br><br>Width (18µm -25µm),<br><br>Thickwalled uniseriate and multicellular<br><br>Trichomes are suberised |
| <b>Glandular hairs</b>       | Rarely present  | Absent   | Present on the lamina  | Present on the lamina   |
| <b>Epidermis</b>             | Single cubical to rectangular layer with thick cuticle  | Mostly cubical with thick cuticle  | Single layered cubical and less cutinised  | Single layered rectangular to cubical cells with thick tangential and radial walls  |
| <b>Palisade tissue</b>       | Single layered elongated closely packed with chloroplast  | Single layered loosely arranged with less chloroplast compared to <i>G.sylvestre</i>   | Single layered compactly arranged with thick chloroplast   | Single layered closely packed with thick chloroplast content  |
| <b>Spongy tissue</b>         | 5-8 layered. Idioblast containing cluster crystals of Calcium oxalate, Lactiferous canals also present        | 5-7 layered. Idioblast containing crystals and lactiferous cavity  | 7-8 layered, compactly arranged with thick chloroplast   | 10-14 layered spongy with large air cavities containing few cluster of crystals and larger lactiferous cavity   |
| <b>Midrib</b>                | Upper part is concave with collenchymatous cells underneath   | Upper part is concave with collenchymatous cells underneath  | Upper part is slightly dome shaped with 1-2 layers of Collenchymas   | Upper part almost flat parenchymatous cells underneath  |
| <b>Stomatal index values</b> | 21  | 16   | 12   | 17  |
| <b>Pericycle</b>             | Parenchymatous  | Parenchymatous   | Parenchymatous   | Parenchymatous  |
| <b>Vascular bundle</b>       | Phloem surrounds xylem except in the both ends.   | Phloem on both sides of xylem  | Phloem surrounds xylem except in the both ends.  | Amphicribal type- phloem completely covers xylem  |
| <b>Phloem</b>                | Fibre absent, lactifers present   | Fibre absent, lactifers are more   | Fibres are poorly developed, lactifers are more and continuous   | Fibres plenty, lactifers are more   |
| <b>Xylem</b>                 | 12-16 radial rows, tracheids varies from 5-10µm   | 12-14 layers with well developed xylem tracheids varies from 5-10µm  | 20-28 radial rows of xylem   | 25-35 radial rows and it is crescent shaped   |
| <b>Cell inclusions</b>       | Rosette and cluster crystals plenty in parenchymatous cells, Few lactiferous cells                            | Crystals more, Few lactifers   | Crystals and lactiferous cells are plenty  | Rosette crystals are few and lactifers are more   |
| <b>Mesophyll tissue</b>      | Not continuous in the upper region of vascular bundle   | Continuous in the upper region of vascular bundle  | Continuous in the upper region of vascular bundle  | Palisade tissue is almost continuous over the vascular bundle   |

Distinguishing characters are summarized as an anatomical key format of the Genus *Gymnema* as follows:

1. Trichomes are suberised, Glandular hairs are present.....2
2. Trichomes are not suberised, Glandular hairs are absent or rarely present.....3

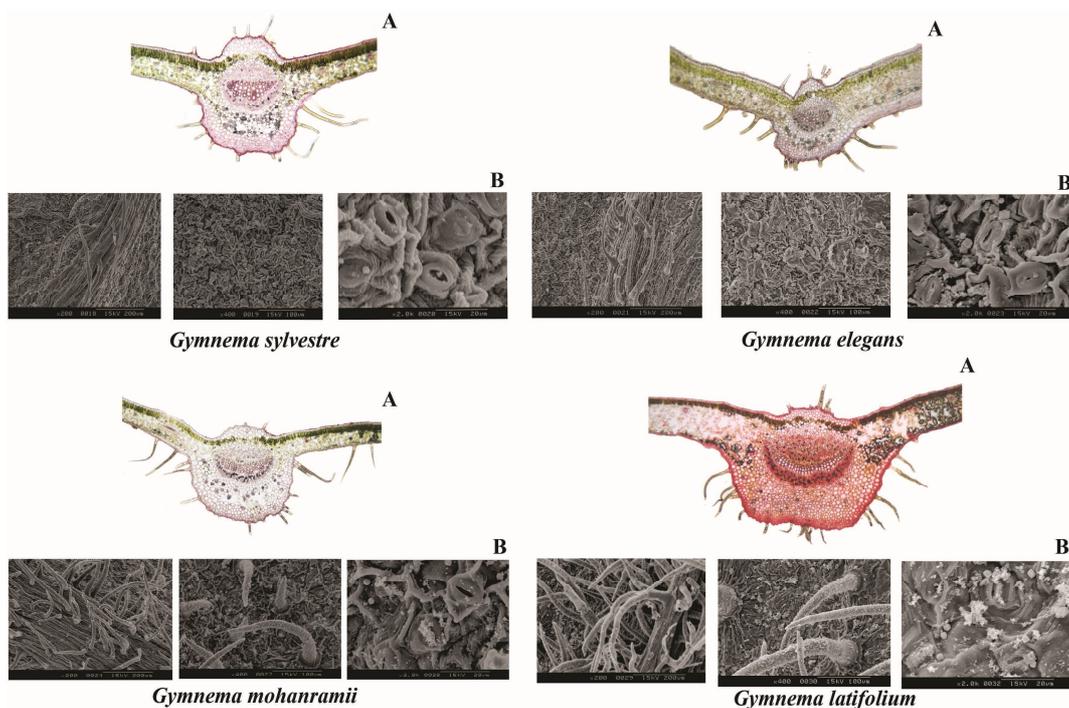
3. Trichomes medium sized length ranges from (50µm - 200µm), epidermis cubical and less cutinised, palisade tissue compactly arranged, spongy tissue 7-8 layered, compactly arranged with thick chloroplast, upper part of midrib slightly dome shaped with collenchymas cells, phloem surrounds xylem of vascular bundle except in the

both ends, phloem fibres are poorly developed, 20-28 radial rows of xylem, mesophyll tissue continuous in the upper region of vascular bundle.....

- .....*G. mohanramii*
4. 2. Trichomes very long and stout (150µm - 400µm), epidermis rectangular to cubical cells with thick tangential and radial walls, palisade tissue closely packed, spongy tissue 10-14 layered spongy with large air cavities containing few cluster of crystals and larger lactiferous cavity, upper part of midrib almost flat with parenchymatous cells, vascular bundle amphicribal type-

phloem completely covers xylem, phloem fibres plenty, 25-35 radial rows of xylem.....*G. latifolium*

5. 3. Trichomes very long (250µm - 450µm), present on mid rib, palisade tissue closely packed with chloroplast, stomatal index values 21, mesophyll tissue not continuous in the upper region of vascular bundle..... *G. sylvestre*
6. 3. Trichomes moderate length (200µm - 300µm), present on mid-rib and lamina, palisade tissue loosely arranged, stomatal index values 16, mesophyll tissue continuous in the upper region of vascular bundle .....*G. elegans*



**Plate 1.** Micromorphological and histological details of genus *Gymnema*.  
A. T.S of Leaf B. Micromorphology of lower epidermis using SEM

The distinguishing characters of the genus *Gymnema* based on their micromorphology showed variability. There is a general relationship between the size of the leaf and trichome frequency. Maximum size of trichomes is observed in *G. latifolium* comparing to other species. The studies were conducted in the epidermal gland of some ferns and made a dichotomous key for the identification of the species [3]. Based on anatomical and histochemical studies on rhizomes, different species of *Curcuma* were classified [4]. In the present study, micromorphological and histological characters of leaf was utilized as a taxonomic tool for inter-species delimitation. A dichotomous key has been prepared using the morphology of trichome characters to identify the four species studied

**4. Conclusion**

The comparative key based on the micromorphology, histology and SEM studies given in this paper is highly useful to distinguish the genus upto species level and to arrive at a conclusion about its correct identity.

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