



Research article

Role of epidermal micromorphology in delimitation of taxa in genus *Andrographis* (Acanthaceae) in India

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Abstract: The leaf epidermal biology of two common medicinal species of genus *Andrographis* in India viz. *A. paniculata* and *A. echoides* were studied in scanning electron microscope. During the study leaf epidermal features such as stomata type, arrangement, size; presence, distribution, size and characteristic features of nonglandular trichomes, glandular trichomes and cystoliths were considered for detail. It has been reported that these two taxa can be clearly distinguished based on these epidermal features. This will aid a good practice for solving complex taxonomic problems in family Acanthaceae.

Keywords: *Andrographis paniculata* - *Andrographis echoides* - Scanning electron microscope - Leaf epidermis.

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INTRODUCTION

The family Acanthaceae is a large pan-tropical family of herbs and shrubs (Scotland *et al.* 1995). Family is represented by more than 3000 species and about 250 genera with its centre of distribution in Africa (including Madagascar), Central America, Indo-Malaysia, Northern South America and Mexico. The family Acanthaceae is considered as a complicated one by most taxonomists as the taxonomical characters amongst species are most diverse. The taxonomic status of genera of family is very complicated and many taxa are yet to be finally delimited. McDade *et al.* (2008) divided the family Acanthaceae into four subfamilies viz. Acanthoideae, Nelsonioideae, Thunbergioideae and Andrographideae. Genus *Andrographis* belongs to subfamily Andrographideae. As per data present on the website of the International Plant Name Index (IPNI), the genus *Andrographis* comprises 40 species. The genus *Andrographis* is of high medicinal value.

Delimitation of taxa based on only morphological characters is tough task in certain genera of many families. Therefore implications of branches such as palynology, embryology, cytology and epidermal micromorphology are nowadays gaining a great importance in solving taxonomic problems. There are many reports on palynological, embryological and cytological studies on family Acanthaceae but studies on epidermal micromorphology based on scanning electron microscopy of the leaf are still rare.

Micromorphological characters of leaves are now gaining great importance for the delimitation of various taxa having identity problems. Many taxonomists have difficulties to identify and classify species in Acanthaceae due to similarities in morphological characters with other species in the same genus. Therefore micromorphological characters of leaves are going to play a very important role in identification of such taxa. Present investigation was carried out to study the leaf epidermal details of two species of genus *Andrographis* i.e., *A. paniculata* (Burm.f.) Nees and *A. echoides* (L.) Nees. The epidermal character included the details of stomata, trichomes, cystoliths, etc.

MATERIALS AND METHODS

Plant sample collection

For the study dried plant material of 2 species of genus *Andrographis* came from the BSA herbarium at Botanical Survey of India, Central Regional Centre, Allahabad, Uttar Pradesh, India.

Leaf sample preparation

Cleaned leaf segments of approximately 10 mm² from each plant samples were mounted on aluminum stubs using two-sided adhesive carbon tapes. These samples were cleaned using fine quality brushes. The samples used in the study were coated with a very thin layer of gold in a sputtering coater and then observed with a scanning electron microscope (Hitachi Table Top 3000). Electron images were recorded for each sample using a digital image processor.

Scanning electron microscopic study

Data were taken for each sample of both species and photographs were recorded. Leaf epidermal features on both adaxial and abaxial surface of leaf viz. stomata type, size, arrangement; presence, distribution and features of nonglandular trichomes; presence or absence of glandular trichomes, their size, frequency and features; cystoliths and its detailed characteristics were considered for the present study.

RESULTS AND DISCUSSION

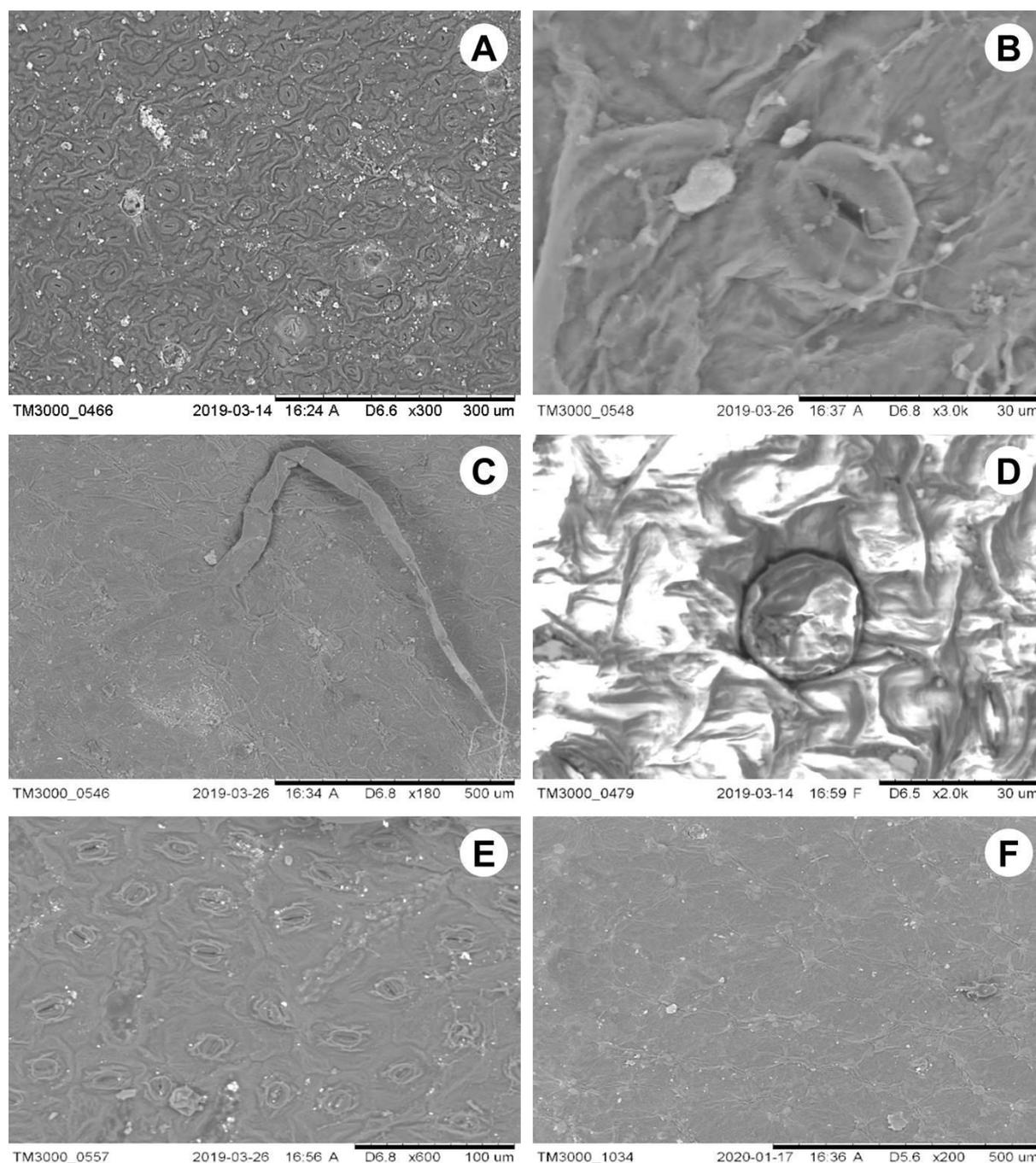


Figure 1. *Andrographis paniculata* (Burm.f.) Nees: **A**, A portion of abaxial epidermis showing distribution of stomata; **B**, Stomata in enlarged view; **C**, Abaxial epidermis showing nonglandular multicellular hair; **D**, A Glandular trichome; **E**, Abaxial leaf epidermis showing cystoliths; **F**, A portion of adaxial epidermis.

The analysis of leaf epidermal studies in *Andrographis* species i.e. *A. paniculata* (Fig. 1) and *A. echioides* (Fig. 2) revealed that epidermal details of two species vary significantly and these species can be clearly delimited based on epidermal features of leaves (Table 1).

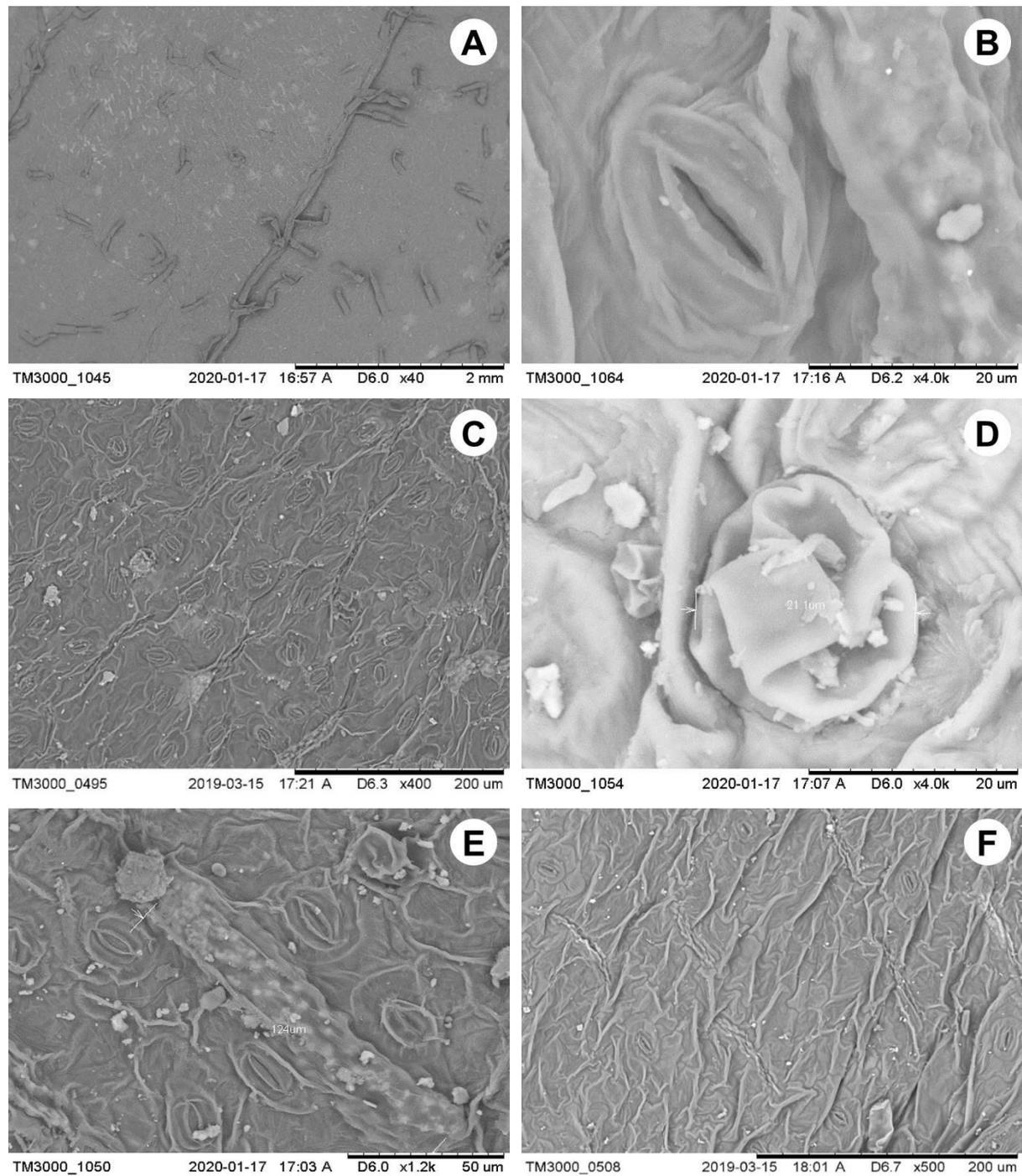


Figure 2. *Andrographis echioides* (L.) Nees: **A**, A portion of abaxial epidermis showing distribution of stomata; **B**, Stomata in enlarged view; **C**, Abaxial epidermis showing nonglandular multicellular hair; **D**, A Glandular trichome; **E**, Abaxial leaf epidermis showing cystolith; **F**, A portion of adaxial epidermis.

The leaves of *A. echioides* are covered with nonglandular hairs and these hairs were very dense in case of the lower epidermis while a few were also observed on the upper epidermis. Unlike *A. echioides* in *A. paniculata* nonglandular trichomes were absent or observed rarely on lower surface. The hairs on the lower surface of leaves of *A. echioides* and significantly large as they can be seen without magnification. The shape of epidermal cells in both species are wavy and cells fitted like tiles.

The leaf of *A. paniculata* and *A. echioides* were hypostomatic and amphistomatic, respectively. The distribution of stomata was irregular in both species and diacytic stomata (The stoma is enclosed by a pair of subsidiary cells whose common wall is at right angles to the guard cells) have been observed in both cases.

Length of stomatal guard cells range from 16.3 μm to 19.1 μm in *A. paniculata* and 18.6 μm to 26.6 μm in *A. echioides*. Breadth of stomatal guard cells range from 14.7 μm to 17.2 μm in *A. paniculata* 13.3 μm to 17.3 μm in *A. echioides*.

Table 1. Comparative leaf epidermal features in *Andrographis paniculata* (Burm.f.) Nees and *Andrographis echioides* (L.) Nees.

Characteristics	<i>Andrographis paniculata</i>	<i>Andrographis echioides</i>
Stomatal features		
1. Stomata type	Diacytic	Diacytic
2. Stomatal arrangement	Irregular	Irregular
3. Stomata guard cell size	Length (μm) min. 16.3, max. 19.1 Breadth (μm) min. 14.7, max. 17.2	Length (μm) min. 18.6, max. 26.2 Breadth (μm) min. 13.3, max. 17.2
4. Presence	Hypostomatic	Amphistomatic and very less on upper surface
Glandular peltate trichome		
5. Shape	Rounded	Rounded
6. Size (mean in μm)	29.4	21.48
7. Frequency	Good on both surface	Dense on lower surface
8. Arrangement	Irregular	Irregular
9. Features	Erupted, sessile, surrounded by rim, circular	Beaded, erupted, like flower, sessile, no rim
10. Presence	Both surface	Both surface but very less on upper surface
Nonglandular trichomes		
11. Frequency	Rare	Good on lower surface, rare on upper surface
12. Shape	Pointed tip	Rounded
13. Arrangement	-	Irregular
14. Features	Multicellular	Multicellular
15. Presence	Rarely on lower surface	Both surface
Cystoliths		
16. Frequency	Good	Dense
17. Diameter (μm)	Min 60.2, Max. 111, mean 89.65	Min. 62, Max. 129, mean 90.875
18. Shape	Rope like knotted, boundaries not clear	Beaded, boundaries clearly demarcated
19. Features	Seen erupted, knotted, appearance	Not erupted, beaded

Glandular trichomes in both species of *Andrographis* are of peltate type. These are rounded in shape and no particular arrangement has been seen in both species. In the case of *A. paniculata* these are present on both surfaces of leaves in good frequency. While in *A. echioides* frequency of these trichomes are good on the lower surface of leaf and rarely a very few have been observed on the upper surface of leaf. In *A. paniculata* these are surrounded by gap appears as rim around these trichomes while in *A. echioides* in appears beaded in structure.

The nonglandular trichomes are multicellular hairs in both species. In *A. paniculata* nonglandular trichomes were seen rarely only on lower surface of leaves while absent of upper surface of leaf. In *A. echioides* these were observed on both surfaces and frequency was very dense on lower surface.

Cystoliths were observed in both species and denser in *A. echioides*. In *A. paniculata* these are rope-like while in *A. echioides* it appears beaded. The mean diameter were 89.65 μm and 90.875 μm for *A. paniculata* and *A. echioides*, respectively.

Leaf epidermal characters are gaining a great importance for solving taxonomic problems in many taxa upto species level. Results of present leaf epidermal study are helpful in solving the delimitation of these two taxa. Both species have similar type of stomata *i.e.* diacytic type. The description of the type of stomata conforms to Metcalfe & Chalk (1979) on their work on the epidermal morphology and stomata types in dicotyledons. These two species can be differentiated on many epidermal characters. Leaves are hypostomatic in *A. paniculata* while in *A. echioides* it was amphistomatic. Glandular trichomes were beaded and flower-like in *A. echioides* while in *A. paniculata* it was somewhat erupted and surrounded by a gap, which appears like a rim around the glandular trichome. Nonglandular trichomes of *A. paniculata* are pointed at end while in *A. echioides* it was blunt-ended.

Trichomes are epidermal in origin and it has diverse characteristic features that can be used in delimitation of various taxa (Werker 2000). However, information on these special structures are still a little know and a wide study in this regard is needed urgently. Metcalfe & Chalk (1950a, b) studied trichomes structures and

diversity in some species and genera of the family. Thus, the present work intends to illustrate types and characteristics features of trichomes in these two taxa of family Acanthaceae. Many workers studied trichomes and they found it useful for delimitations of various taxa of families, for *e.g.* Asteraceae (Krak & Mraz 2008, Angulo & Dematteis 2014, de Andrade Wagner *et al.* 2014), Brassicaceae (Abdel 2005, Beilstein *et al.* 2006), Cucurbitaceae (Ali & Al-Hemaid 2011) Ranunculaceae (Hoot 1991), etc. The size of cytoliths in both species are more or less similar.

CONCLUSION

From the above discussion, it is very clear that in terms of leaf epidermal characters these two species of *Andrographis* can be clearly distinguished. The epidermal characters such as presence and absence of stomata and nonglandular trichomes on adaxial and abaxial surface of leaves, characteristics of nonglandular and glandular trichomes and cystoliths play a very important role in identification and delimitation of these taxa. Studies on leaf epidermal biology based on Scanning Electron Microscopy are still rare in the genus of family Acanthaceae and it is first of its kind of study in these two taxa, which will significantly encourage the studies of leaf epidermis based of scanning electron microscopy for solving various complex taxonomic problems.

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