J. Indian bot. Soc. (2025) Vol. 105 (3): 177-187

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.61289/jibs2025.03.22.0385

www.indianbotsoc.org

RESEARCH ARTICLE

Foliar micromorphology as an additional taxonomic tool to identify taxa of *Abutilon* Mill. (Malvaceae) endemic to Indian Thar desert

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Abstract

The present research work is based on the study of leaf micromorphological variations between two endemic species of genus *Abutilon* namely *A. bidentatum* and *A. fruticosum* distributed in the Indian Thar Desert area of Rajasthan. Leaves of both species are amphistomatic and amphitrichomatic. However differentiation can be made in both the species on basis of epidermal cell wall pattern, stomatal frequency and stomata index, trichome density and morphologically diverse type of egalndular and glandular trichomes. Micromorphological features of leaves can serve as a valuable taxonomic tool for identifying and distinguishing species, even in their vegetative state when flowers and fruits are absent.

Keywords: *Abutilon fruticosum*, guard cell area, micromorphology, peltate gland, stellate trichome

Introduction

The Great Indian Thar Desert is located between latitudes of 24° to 28° North and longitudes of 69° 30 to 78° 17° East (Arora *et al.* 2010). The Thar Desert covers around 60% of Rajasthan's territory, mostly in the districts of Jodhpur, Jaisalmer, Barmer, Bikaner, and Jalore. In spite so many adverse environmental conditions this region is gifted with unique plant diversity in the world. Moreover, the region's vegetation pattern has been impacted by shifting climatic circumstances. Many economically significant species have become endemic due to altered land use patterns, irrigation, and shifting climatic conditions. "Approximately 31 species have converted into vulnerable or endangered. Out of these, 17 species and 18 botanical varieties have become endemic" (Arora *et al.* 2010, Singh, 2004).

Abutilon Mill. is a significant and notably challenging genus of medicinal plants within the Malvaceae family, highlighting the need for a comprehensive and up-to-date revision (Fryxell, 1997). There are over 200 species in the genus *Abutilon*, which is extensively dispersed worldwide (Sivarajan and Pradeep, 1996). It was reported that the unique growth behaviour and ecological impact make Abutilon species a valuable addition to gardens, rather than a threat to local biodiversity (Rankel, 2024). So far eighteen species have been identified from India (Kumar, 2001; Singh 2002). Eight species from Rajasthan have been reported (Shetty and Singh,1987) while the Indian Thar desert area six species of *Abutilon* have been reported namely *A.indicum*(L.) Sweet, *A.pannosum*(G.Forst) Schltdl., *A.ramosum*(Cav.) Guill.&Perr., *A.bidentatum* (Hochst.) A.Rich, *A.fruticosum* Guill & Perr. and *A.pakistanicum* Jafri& Ali (Bhandari,1978;1990) .Out of these, two species *Abutilon bidentatum* Hochst. *var. major* (Blatt. and Hallb.) *Abutilon fruticosum* Guill. and Perr. *var. chrysocarpa* (Blatt. &Hallb.) endemic to this region.

The genus *Abutilon* is important medicinal plant with significant economic value (Hussain & Baquar, 1974). Various plant parts were reported with hypoglycaemic, analgesic,

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How to cite this article: Bano, I., Vandana, Deora, V. Deora, G.S. (2025). Foliar micromorphology as an additional taxonomic tool to identify taxa of Abutilon Mill. (Malvaceae) endemic to Indian Thar Desert. *J. Indian bot. Soc.*, 105(3):177-187 Doi: 10.61289/jibs2025.03.22.0385

Source of support: Nil

Conflict of interest: None.

Received: 22/10/2024 **Accepted:** 22/03/2025 **Published online**: 20/04/2025

hepatoprotective and hyperlipidaemic activity (Ahmed *et al.* 1990; Roshan *et al.* 2008; Ramar and Ayyadurai, 2015). Root and bark of this plant have been used in diabetes and as a nervine tonic (Kirtiar and Basu, 2001). Seeds for the treatment of piles, urinary disorders and cough (Naqshi *et al.* 1988). Some plant species of *Abutilon* also have been reported to contain antimalarial activity (Beha *et al.* 2004).

Before exploration of any plant confirm and correct identification is crucial which can be achieved by morphological features with other supporting tools for identification such as anatomy, micromorphology, embryology, cytology phytochemistry, molecular biology etc.

The use of micromorphology data for certain groups of plants is important to eliminate the uncertainties regarding the classification (Constance, 1964; Stace, 1984).

Under micromorphological studies leaf epidermal features such as shape, size, type and nature of stomata, epidermal appendages like trichomes and hairs, their types, size, structure and type of epidermal cells, leaf venation pattern etc. have been proved to be useful to study the interrelationships between taxa and identification and delineation of species most importantly at generic and species level (Heywood, 1971, Hardin, 1979; Metcalf &Chalk, 1950; Inamdar, 1983; Solereder, 1908,). The importance of epidermal characters hasbeen widely recognized (Hagerup, 1952, Kyungsik *et al*. 1997). The leaf is an organ with considerable variation in anatomical structures, particularly in the epidermal tissue, making it an important source of taxonomic data. (Zhao *et al.* 2022, Gang *et al.* 2021, Shah *et al.* 2018, Ullah *et al.* 2018, Ercan *et al.* 2021). The epidermal cells surrounding the stomata have a tendency to form patterns which were constant within taxa and even families. Stomata and trichomes are widely used for taxonomical studies due to their unique and constant features (Tomlinson, 1974; Wilkinson,1979, Palmer and Jones, 1988).

The primary objective of this study is to provide a detailed description of the micromorphology of two *Abutilon* species endemic to the Indian Thar Desert: *A. bidentatum* and *A. fruticosum*. This research aims to clarify the extent to which micromorphological characteristics can assist in the taxonomic delimitation of these species and to assess the utility of these features for future revision and classification.

Although series of descriptions of the morphological characteristics and ethnobotanical uses of *Abutilon* are documented (Gill and Kaur, 2015; Gomaa *et al.* 2016; Taia, 2009). However, the authors did not come across any information on the epidermal features of the foliar organ of these two species of *Abutilon*.

There is a lack of information on the microscopic characteristics of Abutilon species that could facilitate their recognition and confirmation. Some studies, such as those by Shaheen *et al.* (2009), indicate that *Abutilon* species from Pakistan exhibit variations in micro morphological features compared to their Indian counterparts (Gill and Kaur, 2016; Bano and Deora, 2017). This raises the question of whether geographic regions contribute to significant variations within the species. Understanding these differences could also enhance our knowledge of the taxonomic and phylogenetic relationships within the genus.

This study aims to address the existing gap in knowledge regarding these important medicinal plants. The results will offer essential epidermal information that will significantly aid in clarifying the identity of these taxa. A micro morphological examination of these species will provide insights into distinguishing them based on characteristics such as epidermal cells and stomata, contributing to a comprehensive dataset that can help reduce adulteration in medicinal formulations.

Materials and Methods

Two species of *Abutilon* endemic to Indian Thar Desert namely *A.bidentatum* and *A.fruticosum* have been selected for study of foliar micro morphological variations.

Field visit and collection of plant samples from study area: A field survey for collection of plant materials from various localities of Jodhpur, Jaisalmer and Barmer region of Indian Thar Desert was done during July to September, 2017.Five population sites were selected for each species from the study area (Fig 1). Three plants were collected from each site for both qualitative and quantitative micro morphological studies.

The collected plant specimens were pressed, dried and mounted on herbarium sheets following the standard methods (Smith 1971; Bridson *et al.* 1998) and the voucher specimen were deposited in the herbarium (JAC) of Dept. of Botany, Jai Narain Vyas University, Jodhpur. All the details about studied specimen like localities, height, altitudes, georeference data and vouchers are presented (Table 1).

Plant identification and authentication was done by BSI, Arid Zone regional Centre, Jodhpur as well as available flora and literatures (Bhandari, 1990; Shetty and Singh, 1987, 1991).

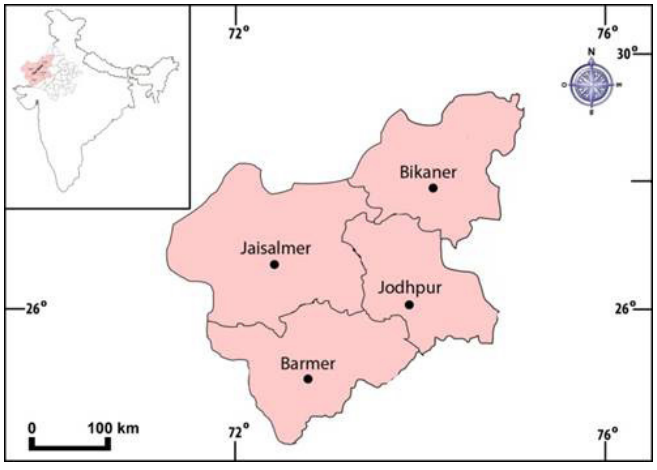

Figure 1: Map of study area with sites of collection

Table 1: List of *Abutilon* species and their site of collection with georeferences

No.	*Name of speciemen collected*	*Localities*	*Longitude °E*	*Latitude °N*	*No. of samples for each location*
1.	*A. bidentatum*Hochst.	Rao Jodha Desert Rock Park and Mehrangarh	73.0167	26.3043°	3
2.	*A. bidentatum*Hochst.	Kiradu hillock, Barmer	71.0977	25.7528	3
3.	*A. bidentatum*Hochst.	RIICO industrial area, Bikaner	73.3402	28.0048	3
4.	*A. bidentatum*Hochst.	Bada Bagh, Jaisalmer	70.8874	26.9553	3
5.	*A. bidentatum*Hochst.	Desert National Park, Jaisalmer	70.8085	26.9191	3
6.	*A. fruticosum* Guill. & Perr.	Mandore Garden, Jodhpur	73.0353	26.3525	3
7.	*A. fruticosum*Guill. & Perr.	Machia Safari Park, Kaylana road, Jodhpur,	72.9763	26.3020	3
8.	*A. fruticosum*Guill. & Perr.	Desert national Park, Jaisalmer	70.8085	26.9191	3
9.	*A. fruticosum*Guill. & Perr.	Wood fossil Park, Akal, Jaisalmer	71.0423	26.8263	3
10.	*A. fruticosum*Guill. & Perr.	Kuldhara village Jaisalmer	70.8030	26.8086	3

Leaf epidermal preparation

For leaf epidermal studies fresh, expanded and fully mature leaves were collected from various localities of study region. Microscopic slides of leaf epidermis were prepared using epidermal peeling and leaf clearing method with some modifications (Leelavathi *et al*., 1980; Bassey *et al.* 2016).The cleared epidermal peeling was stained with 1 % safranine solution for better imaging and then mounted with 10% glycerine.

Both qualitative and quantitative features were examined using a trinocular light microscope at magnifications of 10X and 45X. All measurements were taken manually with an ocular micrometer and adjusted based on the ocular constant corresponding to the magnification used. Data analysis involved calculating the mean value and standard error. Stomatal frequency and stomatal index were determined using the formulas provided by Gupta (1961), Salisbury (1928), and Dilcher (1974).

$$\text{Stomatal frequency (S.F.)} = \frac{S}{E} \times 100$$

$$\text{Stomatal index (S.I.)} = \frac{S}{E+S} \times 100$$

Where S = Number of stomata per unit area of leaf epidermis
E = Number of epidermal cells in the same unit area of leaf epidermis.

The surface area of the guard cell was calculated by multiplying the length and breadth of the guard cell on the adaxial and abaxial surface by the Franco constant (1939). Guard cell area = (length × width × k) µm2 Where, the Franco constant (k) = 0.7852

Trichome density was calculated by counting the number of trichomes per unit area on both leaf surfaces. Basic terminology used in trichomes and stomata suggested by Payne (1978), Inamdar (1983), Haris and Haris (1994), Celka

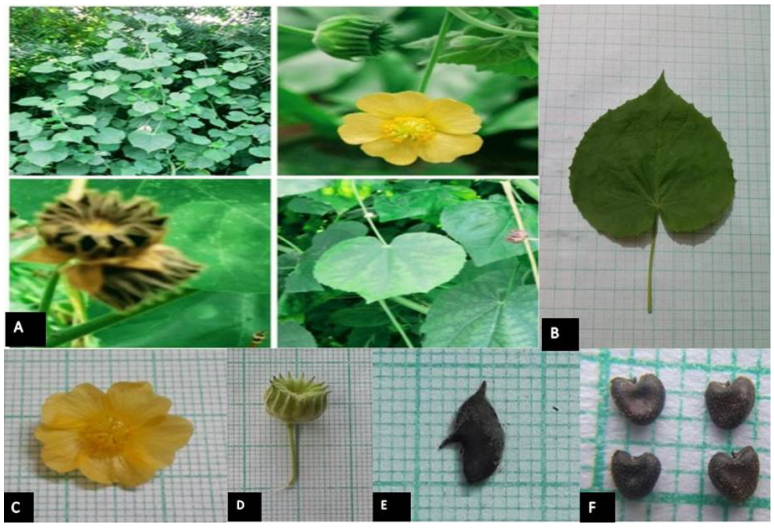

Figure 2:A. Habit of Abutilon bidentatum B. Leaf C. Flower D. Fruit E. Mericarp F. Seeds

et al. (2006) and Shaheen *et al.* (2009). Microphotographs were taken with a Nikon FX-35A camera equipped with a light microscope.

Results

The leaves of *A.bidentatum* hairy, deeply chordate with bidentate leaf margins while the leaves of *A.fruticosum* are comparatively thicker ,velvety, obovate chordate and slightly wavy to entire leaf margins(Fig 2,3). Foliar epidermal micromorphological feature of the investigated species and their qualitative and quantitative analysis are summarized and presented (Table 2,3,4).Selected light microscopic photographs of leaf micromorphological features are shown (Fig.4-5).

The study of micromorphological and architectural parameters of *A.bidentatum* and *A.fruticosum* leaves using light microscopy revealed a number of important features intrinsic to its habitat.

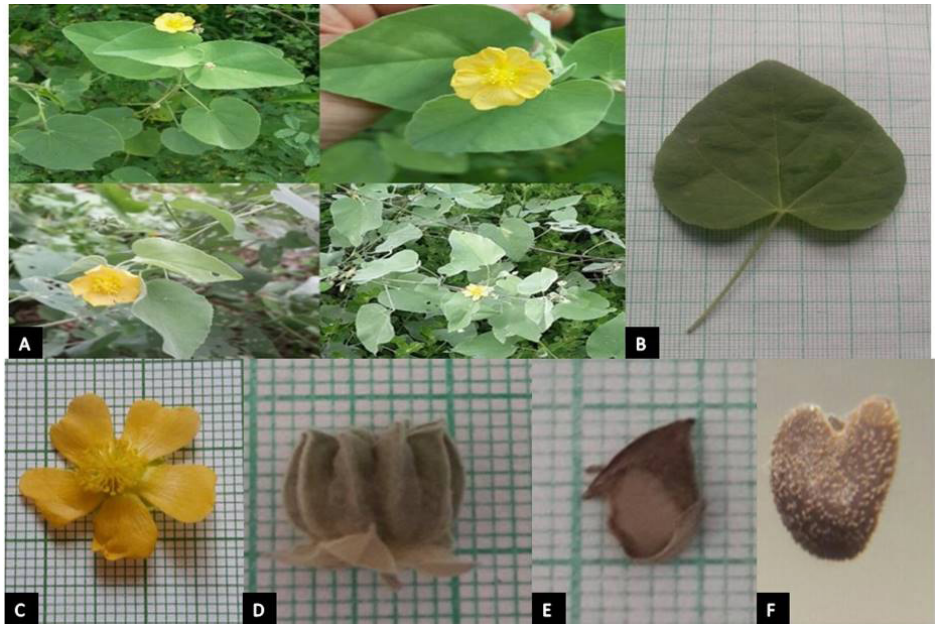

Figure 3:A. Habit of *Abutilon fruticosum* B. Leaf C. Flower D. Fruit E. Mericarp F. Seeds

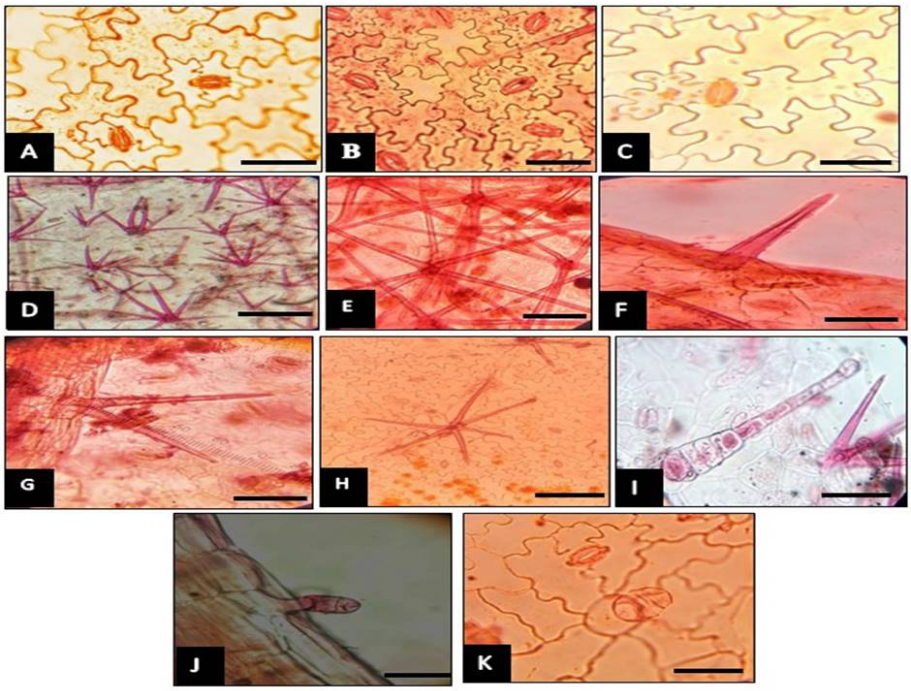

Figure 4: Micromorphological features of leaf epidermis of *Abutilon bidentatum*at450x *magnification*: (Scale bar -50 um): A. Adaxial leaf surface with anisocytic stomata B. Abaxial leaf surface C. Tetracytic stomata D. Trichome density on adaxial leaf surface E. Trichome density on abaxial leaf surface F. Unicellular trichome G.Bifurcated trichome H. Stellate trichome I.Flask shape glands type II J. Capitate gland K. Peltate gland

Table 2: Leaf micromorphological features of two *Abutilon* species

S. N.	*Micromorphological features*	***bidentatum***		***A.fruticosum***	
		Ad	*Ab*	*Ad*	*Ab*
1.	Epidermal cell (L × W) (μm)(Mean ± S.E.)	47.32 ± 1.72 × 19.24 ± 1.72	57.72 ± 3.74 × 19.76 ± 2.41	70.72 ± 3.52 × 27.56 ± 3.34	69.68 ± 9.81 × 36.4 ± 5.87
2.	Epidermal cell density per mm^2 (Mean ± S.E.)	206.65 ± 7.39	265.42 ± 24.29	178.82 ± 10.94	279.99 ± 28.57
3.	Epidermal cell shape	Irregular with undulating wall pattern	Irregular with undulating wall pattern	Polygonal with wavy wall pattern	Irregular with undulating wall pattern
4.	Type of stomata	Anisocytic,Tetracytic	Anisocytic, Tetracytic	Anisocytic Tetracytic stomata	Anisocytic Tetracytic stomata
5.	Subsidiary cell(L × W) (μm)(Mean ± S.E.)	28.94 ± 2.58 × 16.29 ± 4.46	30.85 ± 1.99 × 17.85 ± 2.12	38.81 ± 2.64 × 20.10 ± 1.14	46.62 ± 1.85 × 33.27 ± 6.65
6.	Guard cell of Normal size stomata(L × W) (μm) (Mean ± S.E.)	20.3 ± 1.97 × 7.28 ± 0.52	22.36 ± 1.04 × 5.2 ± 0	22.8 ± 0.97 × 7.8 ± 0	19.76 ± 1.04 × 7.8 ± 0
7.	Guard cell area for Normal size stomata(μm^2) (Mean ± S.E)	110.41 ± 11.56	91.3 ± 4.25	140.13 ± 5.96	21.01 ± 6.37
8.	Guard cell **of** Giant size stomata(L × W) (μm) (Mean ± S.E.)	Absent	Absent	32.24 ± 1.56 × 7.28 ± 0.52	31.72 ± 0.97 × 7.8 ± 0
9.	Guard cell area for Giant size stomata(μm^2) Mean ± S.E	Absent	Absent	234.70 ± 4.5	247.42 ± 6.24
10.	Stomatal frequency per mm^2 (Mean ± S.E.)	6.73 ± 1.05	15.09 ± 1.38	12.36 ± 2.18	20.02 ± 2.17
11..	Stomatal density per mm^2 (Mean ± S.E.)	19.41 ± 6.06	64.68 ± 2.20	22.35 ± 4.32	57.65 ± 9.74

Micromorphology of Epidermal Cells

The epidermal cells on both the adaxial and abaxial surfaces of the leaf of *A. bidentatum,* as well as the abaxial surface of *A. fruticosum*, exhibit irregular shapes with undulating anticlinal wall patterns (Fig. 4A). In contrast, the adaxial surface of A. fruticosum features polygonal-shaped epidermal cells with wavy anticlinal walls (Fig. 5A). Notably, A. fruticosum shows larger epidermal cell sizes and higher epidermal cell density (Table 2).

Micromorphology of Stomata

Both plant species have amphistomatic leaves, with higher stomatal density on the abaxial surface. The mature stomata in both species are of the anisocytic and tetracytic types (Fig. 4C, 5B). However, *A. fruticosum* is characterized by anisocytic stomata with comparatively larger guard cell areas (Table 2). Stomatal dimorphism was frequently observed on the abaxial surface of *A. fruticosum*, with normal-sized stomata (less than 25 μm) alongside larger stomata (greater than 25 μm) (Fig. 5C). *A. fruticosum* exhibited higher stomatal frequency and stomatal index, with both species showing elevated stomatal frequency and index on the adaxial surface (Table 2).

Micromorphology of trichome

The micromorphology of trichome in *Abutilon* species plays a crucial role in species-level identification. Both species under study exhibit amphitrichomic characteristics, with varying trichome densities on both leaf surfaces. In *A. bidentatum*, the trichome density is higher on the abaxial (lower) leaf surface compared to the adaxial (upper) surface (Fig. 4E), whereas *A. fruticosum* shows a higher trichome density on the adaxial surface (Fig. 5D).

Two main types of trichomes were identified in both species: glandular and eglandular. Among the eglandular trichomes, simple unicellular, bifurcated, and stellate types were observed. The glandular trichomes included flask-shaped (Type III) and multicellular uniseriate (Type III), along with capitate and peltate glands in both species (Fig. 4 D-K & 5 D-L). Variations in arm length, width, trichome density, and the number of ray cells in stellate trichomes were noted. The eglandular stellate trichomes of *A. fruticosum* are distinct

from those of *A. bidentatum* due to their shorter length. In *A. bidentatum*, the stellate trichomes possess 3-8 ray cells, while in *A. fruticosum*, they contain 3-6 ray cells (Table 3, 4).

Additionally, *A. bidentatum* features Type II flask-shaped glands (characterized by a basal swollen portion and an upper neck-like structure with a capitate head) (Fig. 4I). In contrast, *A. fruticosum* exhibits Type III flask-shaped glands (with a basal dilated portion gradually narrowing upwards) (Fig. 5I), as well as multicellular uniseriate Type III glands (with a basal swollen portion and an upper neck-like part without a capitate head) (Fig. 5J), a distinctive feature of this species.

Discussion

Abutilon bidentatum and *Abutilon fruticosum* play a vital ecological role in the Thar Desert through soil stabilization, fodder provision, pollinator support, ethno medicinal value and contribution to desert greening efforts. These species are crucial in maintaining the ecological integrity of desert ecosystems, supporting biodiversity, livelihoods, and ecosystem services essential for the sustainability of life in the Thar Desert. As both plant species are endemic to the region and it is necessary that correct discrimination can be made between these species for future conservation efforts on these species. Traditionally, plant identification has primarily relied on morphological characteristics, especially flowers and fruits. However, since flowers and fruits are not present year-round, leaf micromorphology provides a valuable alternative for identifying plants in their vegetative state, offering supplementary diagnostic features. Previous taxonomic studies based on leaf micromorphology have been conducted on various plant families and genera (Solereder, 1908; Metcalfe and Chalk, 1950; Stace, 1984; Inamdar *et al.* 1983, Hafez *et al.* 2017; Johnny *et al.* 2022). Paul *et al.* 2021 studied detailed foliar macro and micro morphological features such as the shape of epidermal cells, stomata, and trichome types and their distribution frequency in 8 Indian genera of Polygonaceae bearing maximum taxa diversity.The observed and analyzed data were found quite unique for each of the studied taxa and significant related to the identification for unconvinced taxa. Song *et al.* 2024 evaluated the potential of micromorphology to distinguish species with taxonomic difficulties within the genus Sanicula(Apiaceae) and provided more additional evidence for exploring the interspecific relationship of the genus.

In the family Malvaceae, stomatal and trichome characteristics have proven to be reliable diagnostic features for distinguishing between different genera and species (Celka, 2006; Shaheen *et al.* 2009; Shaheen *et al.* 2010). Some studies on *Abutilon* species have been carried out in

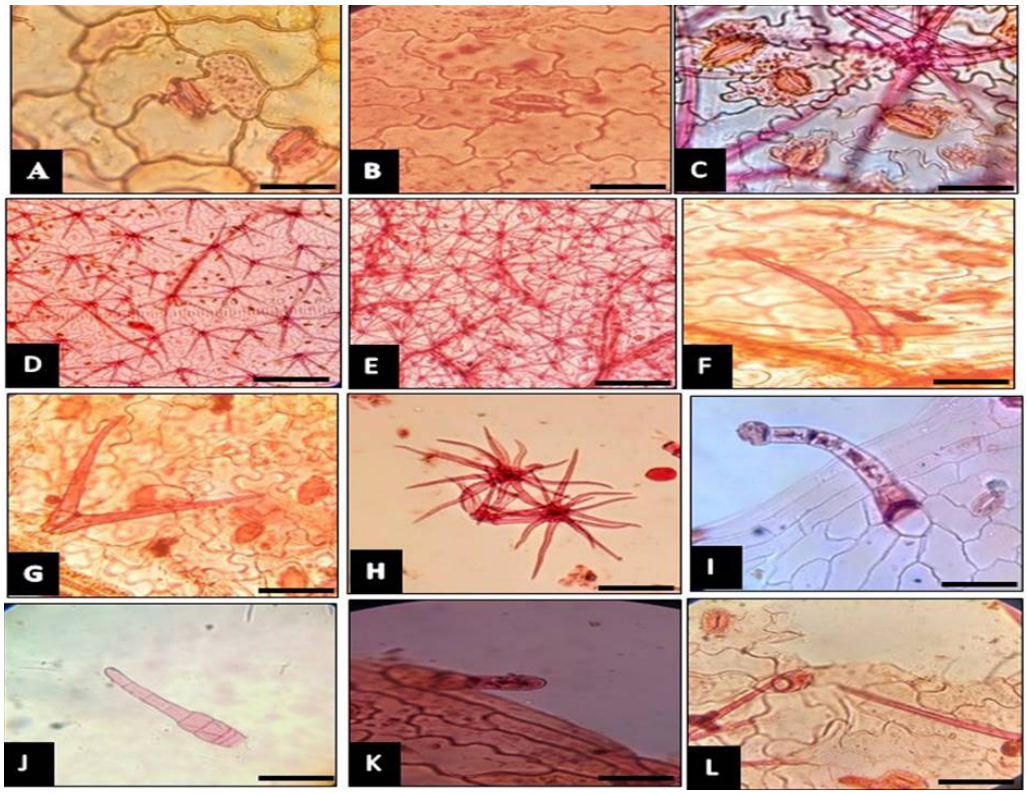

Figure 5: Micromorphological features of leaf epidermis of *Abutilon fruticosumat*450x magnification (Scale bar -50 um):A. Adaxial leaf surface with anisocytic stomata B. Abaxial leaf surface with tetracytic stomata C. Abaxial leaf surface with giant stomata D. Trichome density on adaxial leaf surface E. Trichome density on abaxial leaf surface F. Unicellular trichome G. bifurcated trichome H. stellate trichome I. Flask shape glands-Type III J. Multicellular and uniseriate trichome type II K. Capitate gland L.Peltate gland

Table 3: Micromophological features of leaf epidermal trichomes in two *Abutilon* species

S.N.	*Micromorphological features*	*A.bidentatum*		*A.fruticosum*	
		Ad	*Ab*	*Ad*	*Ab*
1.	Unicellular Conical trichome (L × W) (µm) (Mean ± S.E.)	3.25 ± 11.007 × 5.2 ± 0	92.04 ± 5.96 × 8.32 ± 0.96	127.92 ± 7.13 × 8.32 ± 0.52	116.48 ± 7.41 × 7.8 ± 0.52
2.	Bifurcated/forked tricome(L × W) (µm) (Mean ± S.E.)	82.767 ± 9.71 × 4.33 ± 2.69	108.68 ± 3.97 × 5.2 ± 0	125.32 ± 5.54 × 7.8 ± 0	105.04 ± 8.32 × 7.8 ± 0
3..	Stellate trichome(L × W) (µm) (Mean ± S.E.)	125.84 ± 13.49 × 7.8 ± 0	158.6 ± 17.15 × 5.2 ± 0	93.4 ± 14.09 × 7.8 ± 0	101.97 ± 9.28 × 7.8 ± 0
4.	Number of ray cells in stellate trichomes	3-8	3-8	3-6	3-6
5.	Trichome density(L × W) (Mean ± S.E) per mm^2	37.25 ± 5.18	47.05 ± 3.39	56.86 ± 5.19	47.06 ± 3.39
6.	Flask shape glands Type II(L × W) (µm) (Mean ± S.E.)	115.96 ± 10.4 × 16.12 ± 2.52	135.2 ± 3.18 × 15.08 ± 0.97	Absent	Absent
7..	Flask shape glands Type III (L × W) (µm) (Mean ± S.E.)	- Absent	- Absent	111.28 ± 6.85 × 12.48 ± 0.64	131.04 ± 6.61 × 11.44 ± 0.636
8.	Peltate glands (L × W) (µm) (Mean ± S.E.)	21.84 ± 0.636 × 20.28 ± 1.27	21.32 ± 0.97 × 21.32 ± 0.972	25.48 ± 2.23 × 20.28 ± 0.52	24.44 ± 1.32 × 19.78 ± 1.97
9.	Capitate glands (L × W) (µm) (Mean ± S.E.)	22.36 ± 0.636 × 21.84 ± 1.04	24.96 ± 0.636 × 21.32 ± 0.97	37.96 ± 4.24 × 23.4 ± 0.82	27.56 ± 1.04 × 22.36 ± 1.33
10.	Multicellular uniseriate glands Type III (L × W)(µm) (Mean ± S.E.)	- Absent	- Absent	112.32 ± 2.65× 18.2 ± 0 -	Absent

Table 4: Diversity, distribution and qualitative features of trichomes in *Abutilon* spp.

S.No.	*Type of trichomes*	*Description*	*Distribution*
1.	Unicellular/Conical trichome	Non glandular trichomes, broad at base, narrow and tapering above.	Present in both the investigated taxa on both the leaf surfaces
2.	Bifurcated/Forked trichome	Two ray cells held together in the same cell cavity	Present in both investigated taxa on both the leaf surfaces
3.	Stellate trichome	More than two ray cells held together in the same cell cavity,	Present in both species studied with variation in size,number of ray cells,and density on adaxial and abaxial surfaces
4.	Flask shape glands	Multicellular and uniseriate glands with broad or swallon base and narrow apical portion with capitate head.	
	Type II	Basal 3-4 celled swallon portion and upper narrow 3-4 celled neck like portion	*A.bidentatum*
	Type III	Flask like glands with broader base gradually narrowing upwards.	*A. fruticosum*
5.	Capitate glands	Glands with simple unicellular club shape head and 2-3 celled stalk.	Present in both species
6.	Peltate glands (Type-II)	Unicellular and cup shape glands with wide opening surrounded with epidermal cells.	Present in both species
7.	Multicellular and uniseriate glands(Type III)	A broad basal 4-5 celled portion and upper beak like elongated portion without capitate head.	*A. fruticosum*

Pakistan (Shaheen *et al.* 2009), the species from India's Thar Desert, such as *A. indicum*, *A. pannosum*, and *A. ramosum*, have shown variations in their leaf micromorphological traits (Bano and Deora, 2017). In the current study, two species—*A. bidentatum* and *A. fruticosum*—were examined, and light microscopy revealed differences in both trichomes and stomata compared to earlier findings (Shaheen *et al.* 2009).

Epidermal cell characteristics serve as valuable supplementary evidence for plant identification. When combined with information on stomata and trichomes, they can provide important insights for taxonomic studies. In the current research, the epidermal cells of *A. bidentatum* and *A. fruticosum* exhibited notable differences in shape, size, and density on both leaf surfaces. *A. bidentatum* showed

greater epidermal cell length, width, and density compared to *A. fruticosum*.

In earlier studies from Pakistan, the epidermal cells of *A. bidentatum* and *A. fruticosum* were reported to have a polygonal shape (Shaheen *et al.* 2009). However, in the Indian Thar Desert, *A. fruticosum* exhibited polygonal epidermal cells on the adaxial surface, while the abaxial surface showed irregular cells with wavy anticlinal walls. In contrast, *A. bidentatum* had irregular epidermal cells with an undulating wall pattern on both leaf surfaces.

In both species studied, anisocytic stomata were the most commonly observed, along with some tetracytic stomata. The distinction between *A. fruticosum* and *A. bidentatum* can be made based on guard cell size, stomatal frequency, and stomatal index, all of which are higher in *A. fruticosum*, making these characteristics useful for species identification. A key diagnostic feature for *A. fruticosum* is the presence of giant stomata on the adaxial leaf surface, which is reported for the first time in this species of *Abutilon*, where only normal-sized stomata had previously been observed. Similar excessively large stomata were earlier noted in the leaves of *Mangifera indica* L. and *Limonia acidissima* L. (Sitholey and Pandey, 1971), as well as on several tropical tree species (Boldt and Rank, 2010).

Giant stomata were typically larger than regular ones and mostly located along the vein of leaves. The size of normal stomata is less than 25 µm while giant stomata size remains more than 25 µm (Carr, 1990; Mitra *et al.* 2015).

A helpful measure for comparing the two taxa could be the stomata frequency and stomatal index value.Since the stomatal index is independent of the environment, size, or percentage of the leaf surface, it is a reliable criterion for identification. (Metcalfe and Chalk, 1979; Oberemi& Oladele, 2001)). The role of the stomatal index in taxonomy is to separate species has also been reported by Abdulrahman and Oladele (2003). The variation in stomatal index observed in these studies can be reasonably employed in delimiting the species of *Abutilon*.

The highest stomatal frequency and stomatal index were found on the adaxial leaf surface of *A. fruticosum* while stomatal density for *A. fruticosum* was recorded lower than *A.bidentatum*. Many researchers reported that stomatal density decreases under drought stress (Mc Cree and Davis, 1974; Yang and Wang, 2001; Zhang *et al.* 2006; Xu and Zhou, 2008). The lesser stomatal density could be because of the drought stress tolerance of *A. fruticosum*.

Although variations in epidermal and stomatal characteristics were observed, they alone were not significant enough for taxonomic differentiation between species. However, when combined with leaf trichome micromorphology, these features provide substantial taxonomic evidence for distinguishing species. The most intriguing aspect of this study was the extensive variety of trichomes found on the leaf surfaces. Trichomes, which are epidermal appendages of leaves, vary greatly in structure and function (Uphof, 1962) and play a crucial role in helping plants cope with various abiotic and biotic stresses (Levin, 1973).

Trichomes are generally classified into two groups: glandular and non-glandular (eglandular). Eglandular trichomes form a protective covering on the plant surface, shielding the plant from predators, UV radiation, excessive light, and water loss, especially in arid environments (Tattini & al., 2007). It has been reported that trichome variations in form and function can be either intraspecific or interspecific (Levin, 1973). In *Abutilon*, trichome micromorphology is an important taxonomic feature for identifying and differentiating species. The *Abutilon* species studied were amphitrichomic, with trichomes densely covering the abaxial leaf surface.

Seven different types of trichomes were observed in both species, categorized as either glandular or eglandular. These trichomes were further subdivided based on species-specific variations, as previously studied by Shaheen *et al.* (2009, 2010). Although morphologically similar, slight differences were noted in flask-shaped trichomes. Stellate trichomes varied in distribution, the number of ray cells, and the relative arm length and width across the species.

In *A. bidentatum*, the number of ray cells ranged from 3–7, while *A. fruticosum* exhibited 3–6 ray cells. *A. fruticosum* had shorter stellate trichomes on the abaxial surface, differing from Shaheen *et al.* (2009) earlier report of longer trichomes in the same species. Additionally, *A. fruticosum* had a higher trichome density on the adaxial surface, forming a thick indumentum. Both species exhibited capitate and peltate glands, with slight variations in size. Interestingly, while Shaheen *et al.* (2009) reported the absence of capitate trichomes in *A. fruticosum*, the present study confirms their presence.

The peltate glands, disk- or cup-shaped and surrounded by an epidermal cell sheath, were also observed. *A. fruticosum* had Type III flask-shaped glandular trichomes, which were multicellular and uniseriate with a capitate head consisting of 8-12 cells, slightly dilated at the base and narrowing upwards. Similar trichomes had previously been recorded in *A. indicum* and *A. pannosum* (Bano and Deora, 2017), as well as in *A. molle* and *Hibiscus trionum* (Shaheen *et al.* 2009). In *A. bidentatum*, Type II flask-shaped glands were observed, with a 3-5 celled basal swollen portion and a 2-4 celled neck-like portion, consistent with previous findings from Pakistan (Shaheen *et al.* 2009).

A. fruticosum can be distinguished from other species of the genus by the presence of multicellular, uniseriate Type II trichomes, in addition to Type III flask-shaped glands, which are reported for the first time in this plant. These trichomes differ from Type II flask-shaped glands by the absence

of a capitate head. The present study also noted that *A. fruticosum* had 3–6 ray cells, contrary to earlier reports of 8–9 ray cells in this species (Shaheen *et al.* 2009).

Thus the trichome characteristics of each *Abutilon* species studied were unique in their anatomical measurements, making them valuable for species identification. This study highlights key stomatal and trichome features that can aid in the taxonomic identification of *Abutilon* species, demonstrating the promise of such research in delineating species within the genus.

Conclusion

Present study reveals that noteworthy variations were observed in leaf epidermal micromorphology between *Abutilon bidentatum* and *A. fruticosum* from Indian Thar desert. Change in geographical variation could lead to these variations between species as well as variations were also reported within species growing in different regions as per the available literature. Although differences observed in epidermal cells are less specific but stomatal index, stomatal frequency and trichome characteristics of both species are so specific that individual species can be identified and delineated on the basis of these features.

A diagnostic key prepared for the two species as follows:

Giant stomata present, multicellular and uniseriate trichome (type III) present, flask shape glandular trichome without capitate head, stellate trichome shorter, number of ray cells less than 5 in stellate trichomes.....*A. fruticosum*

Giant stomata absent, multicellular unseriate trichome type III absent,flask shape glandular trichome with capitate head……*A.bidentatum*

Author Contributions

Author 1: Ilham Bano Worked on Conceptualization, methodology, investigation, data curation, formal analysis, and writing—original draft preparation.

Author 2 Vandana and Author 3 Vinod Deora: Data collection, resources management, and visualization.

Author 4 G.S.Deora: Supervision, validation, review, and editing of the manuscript, Statistical analysis, and data interpretation.

All authors have read and approved the final version of the manuscript.

Acknowledgements

Authors are thankful to the University Grant Commission, New Delhi for providing financial assistance in form of scholarship to one of the authors and through CAS programme in the Department of Botany, Jai Narain Vyas University, Jodhpur (Rajasthan) India.

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