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(Research Article)

Influence of stomatal traits on the ecological classification of selected plants in the lateritic belt

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Abstract

Stomatal morphology and distribution exhibit remarkable diversity across monocot and dicot species, reflecting their evolutionary adaptations to varied ecological niches. This study investigates the stomatal diversity in selected taxa, highlighting its taxonomic significance and functional implications. In monocots, the presence of brachyparacytic stomata in *Acorus calamus* represents a primitive trait, while the tetracytic condition in *Kaempferia galanga* and *Tradescantia spathacea* suggests advanced gas exchange mechanisms. Hypostomatic species like *Costus speciosus* and *Tradescantia spathacea* indicate adaptations for water conservation, whereas amphistomatic taxa such as *Acorus calamus*, *Caladium bicolour, Dracaena marginata,* and *Kaempferia galanga* thrive in humid environments. *Dracaena marginata* and *Polianthes tuberosa* exhibit anomocytic stomata, linking them to basal monocot lineages. The unique dumbbell-shaped guard cells of *Cynodon dactylon* exemplify Poaceae's optimization of transpiration control.

Dicot species exhibit a spectrum of stomatal adaptations aligned with their ecological conditions. *Bryophyllum calycinum* demonstrates anisocytic stomata along with CAM metabolism, enhancing water-use efficiency. *Calotropis procera* displays diverse stomatal types, suggesting specialized xeric adaptations. Anomocytic stomata in *Euphorbia neriifolia*, *Rauwolfia serpentina*, and *Tabernaemontana divaricata* indicate a basal evolutionary trait balancing transpiration. Hypostomatic conditions in *Ficus microcarpa* and *Rauwolfia serpentina* promote water conservation, while sunken stomata in *Nerium oleander* and *Ficus microcarpa* reduce transpiration.

Additionally, stomatal multiplicity in both dicots and monocots were observed. Novel types, including laterocytic, stephanocytic, diacytic, cyclocytic, amphipseudoholoparacytic, stomata were also reported. Scanning electron microscopy revealed sunken stomata and non-contiguous stomatal clusters. These findings underscore the interplay of genetic and environmental factors in stomatal development. Further molecular and physiological studies will help us to understand how stomatal diversity evolved and adapted. This will improve our knowledge of plant responses to climate change and water-use efficiency.

Keywords: Stomatal Diversity; Evolutionary Adaptations; Taxonomic Significance; Environmental Factors; Ecological Condition

1. Introduction

Stomata, the microscopic pores on plant leaf surfaces, play a pivotal role in regulating gas exchange between plants and the atmosphere [1]. This exchange is essential for photosynthesis, where carbon dioxide (CO_2) is taken up, and in transpiration, where water is released [2]. The efficiency of these processes is significantly influenced by stomatal

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density (SD), stomatal index (SI), and stomatal morphology as key determinants of plant adaptation to diversified environmental condition [3].

Stomata first emerged between the late Silurian and early Devonian periods [4], marking a crucial milestone in plant evolution. According to few scientists [4], the fundamental structure of stomata has remained remarkably consistent over approximately 400 million years, making it one of the most evolutionarily stable features of land plants. Among modern plants, the earliest stomata are observed in Bryophyta, though they appear only in the sporophytic stage [5]. Vascular plants universally possess abundant stomata [6], with grasses exhibiting the most advanced and efficient form—characterized by their distinctive dumbbell shape.

The relationship between stomatal traits and environmental factors is crucial for ecological grouping, which involves classification of plants based on their functional adaptations and ecological roles [7]. Plants adjust stomatal size and density to adapt to different moisture, temperature and light regimes [3]. By analyzing these adjustments, it can be achieved the procedure of obtaining plant’s water use efficiency (WUE) and carbon recycling in response to environmental pressures [2].

Stomatal density is the number of stomata per unit area, and it is a critical parameter affecting the rate of gas exchange. Higher stomatal density can potentially increase CO_2 uptake for photosynthesis, but it also raises the risk of water loss through transpiration [8]. Therefore, plants in water-stress condition often exhibit lower stomatal densities as an adaptation to conserve water.

Stomatal index (SI) represents the proportion of stomata relative to epidermal cells and provides a normalized measure of stomatal abundance. This index helps to account for variations in epidermal cell size, offering a more robust comparison of stomatal frequency across different species or environmental conditions [9].

Stomatal morphology, including pore size and shape, also influences gas exchange efficiency [1]. Smaller, more numerous stomata can respond more quickly to environmental changes, permeating for finer control over transpiration and CO_2 uptake. On the other hand, larger stomata may facilitate higher gas exchange rates under favourable conditions [8].

Light intensity and quality are significant factors influencing stomatal development and function. Plants grown under high light intensity often exhibit higher stomatal densities to support increased photosynthetic rates [2]. The spectral composition of light also plays a role, with blue light, for instance, affecting stomatal formation and nutrient absorption in plants [10].

Water availability is a primary driver of stomatal adaptation. In drought-prone environments, plants tend to develop lower stomatal densities and smaller stomatal sizes to minimize water loss. The ability to close stomata rapidly in response to water stress is also a crucial adaptation for survival in arid conditions [11]. Carminati *et al.*,2020 [12] showed that soil water availability is a key factor in controlling stomatal response to drought. Atmospheric CO_2 concentration also influences stomatal development [13]. Elevated CO_2 levels can lead to decreased stomatal density, as plants require fewer stomata to capture sufficient CO_2 for photosynthesis. This response can have implications for plant water use efficiency and overall ecosystem productivity. Ecological grouping based on stomatal traits involves classifying plants into functional groups that share similar adaptations to specific environmental conditions [4]. This approach helps to understand the ecological roles of different plant species and predict their responses to environmental changes [14]. Plants can be broadly classified into hydrophytes (aquatic plants), mesophytes (plants in moderately moist environments), and xerophytes (plants in arid environments). Hydrophytes often have fewer stomata, primarily on the upper leaf surface, while xerophytes exhibit adaptations such as sunken stomata and thick cuticles to reduce water loss. Mesophytes typically have a moderate stomatal density and distribution, reflecting their adaptation to balanced water availability. Understanding the extent to which plants can modify their stomatal traits in response to short-term environmental changes is essential for predicting their resilience to climate change [10]. Studies on stomatal plasticity and acclimation can reveal the limits of adaptation and identify species that are more vulnerable to environmental stress.

Developing mechanistic models that integrate stomatal physiology, morphology, and environmental factors is crucial for predicting plant responses at larger scales [14]. These models can help to assess the impact of climate change on plant productivity, water use efficiency, and ecosystem dynamics. Considering the above views, the present work has been done with the following Objectives:

- To Analyze Stomatal Adaptations: Explore how stomatal density, index, and morphology contribute to the adaptation of plants in lateritic soil conditions.
- To Understand Ecological Grouping: Examine the role of stomatal traits in classifying plants into specific ecological groups within the lateritic belt.
- To Investigate Environmental Responses: Study how stomatal characteristics influence plant responses to abiotic stresses typical of lateritic regions, such as low water retention and nutrient deficiency.
- To Support Conservation Efforts: Provide insights into the ecological roles of plant species in lateritic ecosystems to aid in biodiversity conservation.

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2. Materials and Methods

2.1. Plant material

Leaves from 7 dicotyledons and 8 monocotyledons species (Table 1) were collected randomly during the present investigation from plants grown in Lateritic belt of Birbhum District, West Bengal, India.

Table 1 Plant Species Selected for the study

Monocotyledons		**Dicotyledons**	
Species	Family	Species	Family
Acorus calamus L.	Araceae	*Bryophyllum calycinum* Salisb.	Crassulaceae
Caladium bicolor (Aiton) Vent	Araceae	*Calotropis procera* (Aiton) W.T.Aiton	Asclepiadaceae
Costus speciosus (Koen. ex. Retz.) Sm.	Costaceae	*Euphorbia neriifolia* L.	Euphorbiaceae
Dracaena marginata V and. ex L.	Asparagaceae	*Ficus microcarpa* L.	Moraceae
Kaempferia galanga L.	Zingiberaceae	*Nerium oleander* L.	Apocynaceae
Polianthes tuberosa L.	Amaryllidaceae	*Rauwolfia serpentina* (L.) Benth. ex Kurz ·	Apocynaceae
Tradescantia spathacea Sw.	Commelinaceae	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	Apocynaceae
Cynodon dactylon (L.) Pers.	Poaceae		

2.2. light microscopy

Leaves are labelled in different microscope slides. Young and mature leaves were fixed in FAA (Formalin-Acetic-Alcohol) and stored in 70% ethanol. Epidermal peels were taken from the central leaf portion. Dry specimens were boiled in 5% KOH for 15 minutes before peeling. Peels were stained with 1% safranin in 50% ethanol and mounted in 50% glycerol. Data was based on 3 observations; stomatal size was measured from 4 observations on 4 samples per taxon. Stomata area was calculated as πr^2 ($\pi = 3.14$, r = mean radius of the field). Observed under LABOMED VISION 2000 L.E.D compound light microscope at 100x or 400x magnification. For leaves with thick waxy deposits, the waxy layer was removed by treating the leaves with acetone or chloroform before applying the replica method. The methodology involves calibrating the ocular micrometer and then using it to measure Stomatal density, stomatal index and length & breadth of stomatal aperture etc.

2.3. Calibration of Ocular Micrometer

The calibration process is essential to ensure accurate measurements. The following steps are involved:

Stage Micrometer Placement: A stage micrometer (SM), which has a calibrated scale, is placed on the microscope stage. A typical stage micrometer has 100 divisions within 1 mm, so each division equals 0.01 mm (10 µm).

The stage micrometer is focused under a specific magnification (e.g., 40x). An ocular micrometer (OM) is placed in one of the microscope's eyepieces. While observing through the eyepieces, the scale of the ocular micrometer is aligned with the scale of the stage micrometer. The number of divisions on the ocular micrometer that coincide with a known number of divisions on the stage micrometer is counted. We found 7.0 divisions of the ocular micrometer align with 10 divisions of the stage micrometer, then 1.0 OD divisions = 10/7X0.01mm =0.0143mm.

2.4. Stomatal Density

The number of stomata within a known area (defined by the ocular micrometer) is counted. Multiple fields of view were counted, and the average stomatal density was calculated as stomata per mm^2.

2.5. Stomatal Index

The number of stomata and the total number of epidermal cells (including stomata) within a known area were counted. The stomatal index is then calculated using the formula:

Stomatal Index = Stomata / (Epidermal cells + Stomata)X100

2.6. Stomatal and Cell Dimensions

The length and width of stomatal apertures, guard cells were measured using the calibrated ocular micrometer. Multiple measurements were taken for each structure, and the average dimensions were calculated.

2.7. Stomatal and epicuticular wax morphology visualized by scanning electron microscopy (SEM)

Field Emission Scanning Electron Microscopy (FESEM) was used to study the topography of the objects, and it worked with electrons rather than light sources (Fig.9). The dehydrated isolated epidermal peels from different plant species collected from field survey were observed under a FESEM (Zes Gemini 2, Germany) after a gold coating using an ion sputter (Quorum Sputter Coaters and SEM and TEM Carbon Coaters, UK). The field emission source generates electrons, which were accelerated toward a high electrical field gradient.

2.8. Study Area

The study was conducted in the Rampurhat sub division, located in the lateritic belt of Birbhum District, West Bengal (Fig.1). The area is characterized by seasonal climatic variations and diverse vegetation, including dicot and monocot plants.

2.9. Statistical analyses

All data were analyzed statistically and standard error (SE) were measured as per Panse and Sukhatme (1967) [15]

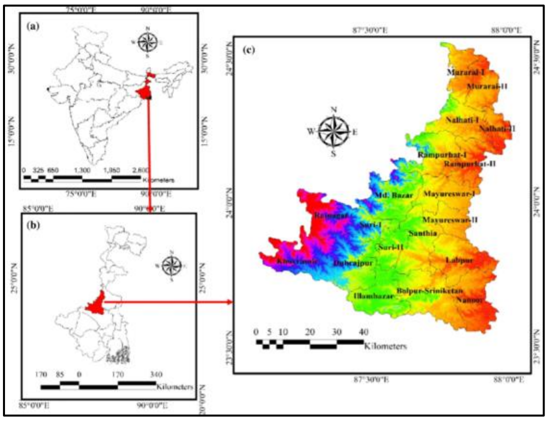

Figure 1 Rampurhat Sub division of Birbhum, West Bengal

2.10. Identification of stomatal complexes

We categorized the types of stomata, their distribution patterns, and their orientation based on the available literature [1,7,23,]

3. Results and discussion

3.1. Monocots plant material

The monocot taxa studied (Fig. 2) along with their stomatal features were summarized in Table 2. The arrangement of the families and the taxa under each family is described alphabetically.

The diversity of Monocot taxa studied was displayed in fig. 4 and the stomatal density and stomatal index was graphically represented in fig. 3.

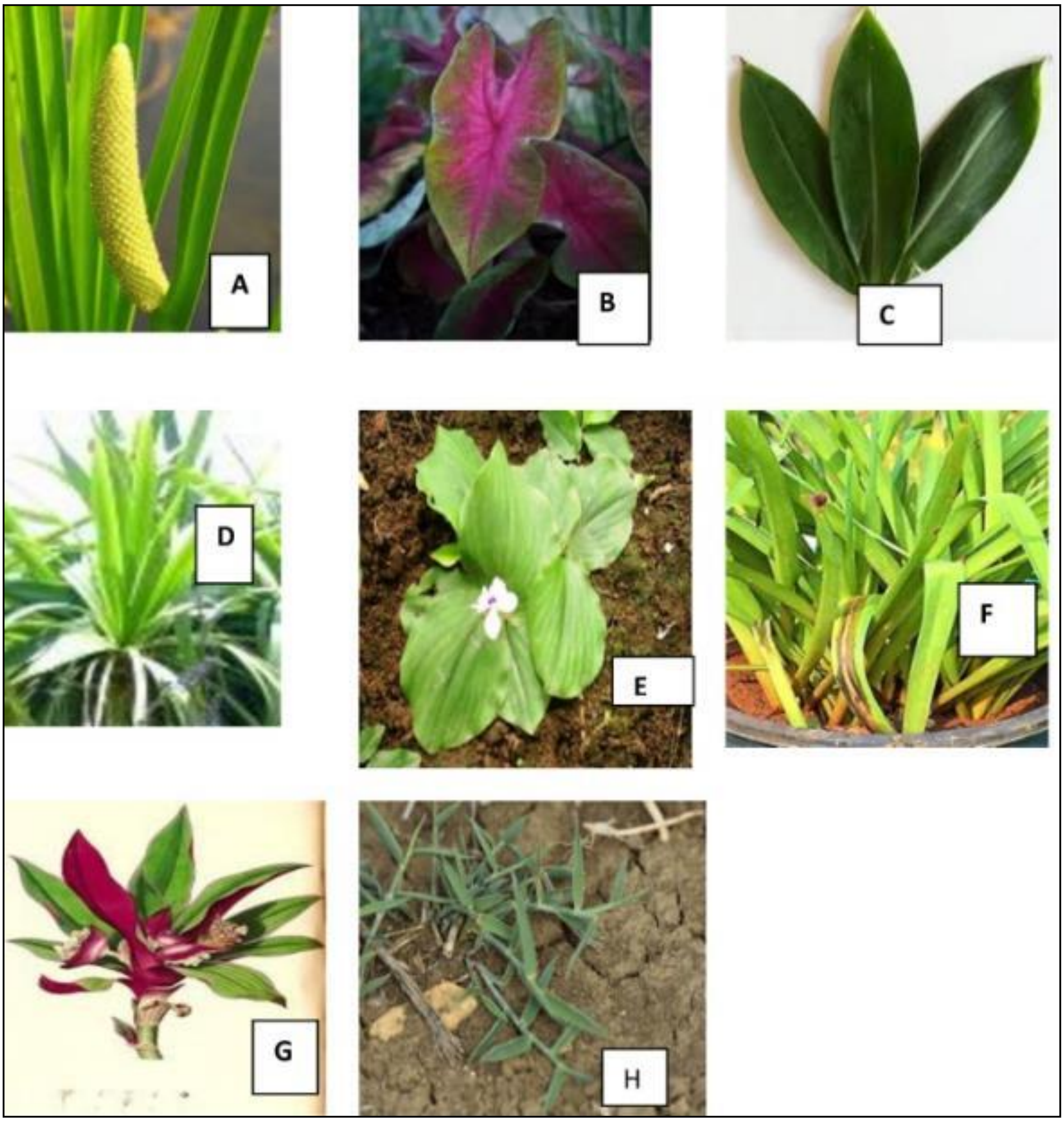

Figure 2 Leaf shapes in some monocots with their family

[(A) *Acorus calamus* (Araceae) , (B*) Caladium bicolor* (Araceae) , (C) *Costus speciosus* (Costaceae) (D) *Dracaena marginata* (Asparagaceae) , (E) *Kaempferia galanga* (Zingiberaceae) , (F) *Polianthes tuberosa* L. (Amaryllidaceae) , (G) *Tradescantia spathacea* (Commelinaceae), (H) *Cynodon dactylon* (L.) Pers.(Poaceae)]

Table 2 Monocot taxa studied and their stomatal features

No.	Species	Family	Stomata Type	Density (stomata / mm^2) in lower surface	Index (%) in lower surface	Adaptation	Group
1	*Acorus calamus*	Araceae	Brachyparacytic Amphistomatic	384.8±1.2	28.59±1.4	Aromatic adaptations favour moderate water regulation	Semiaquatic
2	*Caladium bicolor*	Araceae	Paracytic Amphistomatic	249.01±1.9	18.5±2.5	Advantageous in shaded, humid environments	Acaulescent herb,
3	*Costus speciosus*	Costaceae	Paracytic, Hypostomatic	273.2±.25	20.3±2.2	The condition in *Costus speciosus* is indicative of advanced specialization	Tropical herb
4	*Dracaena marginata*	Asparagaceae	Anomocytic Amphistomatic	363.4±1.33	27.3±1.3	Xerophytic	Succulent shrubs
5	*Kaempferia galanga*	Zingiberaceae	Tetracytic Amphistomatic	134.2±1.4	10.1±.55	The tetracytic configuration facilitates water-use efficiency and carbon assimilation	Tropical aromatic herb
6	*Polianthes tuberosa*	Amaryllidaceae	Anomocytic Amphistomatic	255±1.77	19.9*±1.33	Wide adaptability	Tropical and temperate herb
7	*Tradescantia spathacea*	Commelinaceae	Tetracytic Hypostomatic	215.09±1.4	16.43±2.6	advanced among stomatal types	Succulent herb
8	*Cynodon dactylon*	Poaceae	Paracytic Amphistomatic	297.87±1.3	22.13±1.6	Adapted in open areas where there are frequent disturbances such as grazing, flooding, and fire.	Warm temperate to tropical herb

3.2. Monocot taxa studied and their stomatal features

The diversity of monocot taxa studied was displayed in Fig. 4 and their diversity and index was graphically represented in Fig 3.

3.2.1. Family: Acoraceae *(Acorus calamus):*

It had been found that the plant was semi-aquatic herb, amphistomatic, brachyparacytic with one pair of subsidiary cells. The guard cells were oriented parallel to the longitudinal axis of the leaf. Epidermal cells were nearly isodiametric in outline. The Brachyparacytic stomata were considered evolutionarily primitive within monocots. The amphistomatic condition was advantageous in shaded, humid environments where light penetration and water availability influenced stomatal functionality and intermediate evolutionary state between terrestrial and aquatic adaptations.

3.2.2. Family: Araceae *(Caladium bicolour)*

The result showed that the plant is a acaulescent herb and stomata were paracytic and amphistomatic. Guard cells were elongated and kidney-shaped with thick inner and outer ledge. Subsidiary cells were almost rectangular with thin walls. Epidermal cells were pentagonal & hexagonal with thick walls. Guard cell contents were prominent and granular materials. The amphistomatic condition was advantageous in shaded, humid environments where light penetration and water availability influence stomatal functionality.

3.2.3. Family: Costaceae *(Costus speciosus)*

The study revealed that the adaxial epidermis of the plant composed of single-layered polygonal cells with paracytic stomata, Abaxial epidermis consised of bluntly angled epidermal cells. The hypostomatic condition in *Costus speciosus* was indicative of advanced specialization. The stomatal type in *Costus speciosus* underscored evolutionary advancements toward optimizing physiological processes in tropical ecosystems.

3.2.4. Family: Asparagaceae *(Dracaena marginata)*

The plant was a succulent shrub, and amphistomatic, anomocytic stomata type i.e. without any subsidiary cells. The anomocytic stomatal type represented a basal state, linking *Dracaena marginata* to early monocot lineages allowing the species to thrive in challenging xerophytic habitats.

3.2.5. Family: Zingiberaceae *(Kaempferia galanga)*

The epidermal cells were large and hexagonal to round in shape. Lamina was amphistomatic, with straight to curved epidermal cell wall. Stomata were tetracytic (four annexed cells per stomatic apparatus) and stomatal index was the highest in abaxial surface of *K. galanga*. The tetracytic stomatal arrangement in *Kaempferia galanga* signified a derived condition compared to simpler stomatal types (e.g., anomocytic or paracytic), showcasing advanced regulatory mechanisms,reflects the adaptation of the species to tropical climates.

3.2.6. Family: Amaryllidaceae *(Polianthes tuberosa)*

It was found that the plant exhibited anomocytic stomata, i.e., the stomata lack distinct subsidiary cells. Scanning electron microscopy (SEM) revealed that stomata were sunken in the epidermis (Fig. 3.7 C). The stomatal type in *Polianthes tuberosa* highlighted the retention of primitive features, demonstrating how plants with basal traits could thrive and adapt in cultivated and natural settings.

3.2.7. Family:Commelinaceae *(Tradescantia spathacea)*

The stomatas were hypostomatic type, which indicated that they were only located on the abaxial face of the leaves of *Tradescantia spathacea*. They were in tetracytic condition. The tetracytic stomata arrangement allowed for dynamic stomatal movement, enabling rapid response to environmental fluctuations.

3.2.8. Family:Poaceae *(Cynodon dactylon)*

The study revealed that abaxial intercostals long leaf cells had thin, sinuous walls. The guard cells were dumbbell-shaped, while the subsidiary cells ranged from low to high dome-shaped. The number of rows of long cells between two costal zones varied from 6 to 10, whereas the number of stomatal rows between two costal zones ranged from 1 to 3.

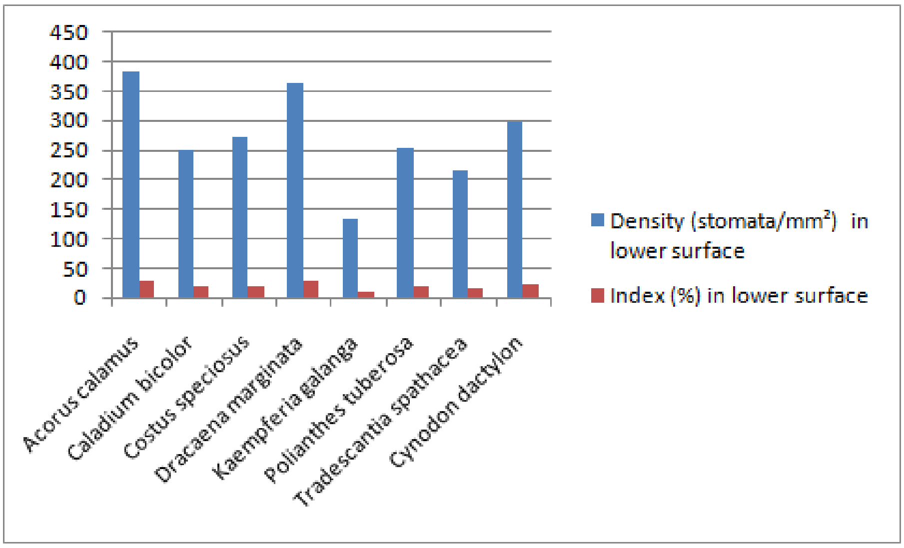

Figure 3 Monocot taxa studied and their stomatal features in graphical representation

3.3. Monocot taxa stomata types

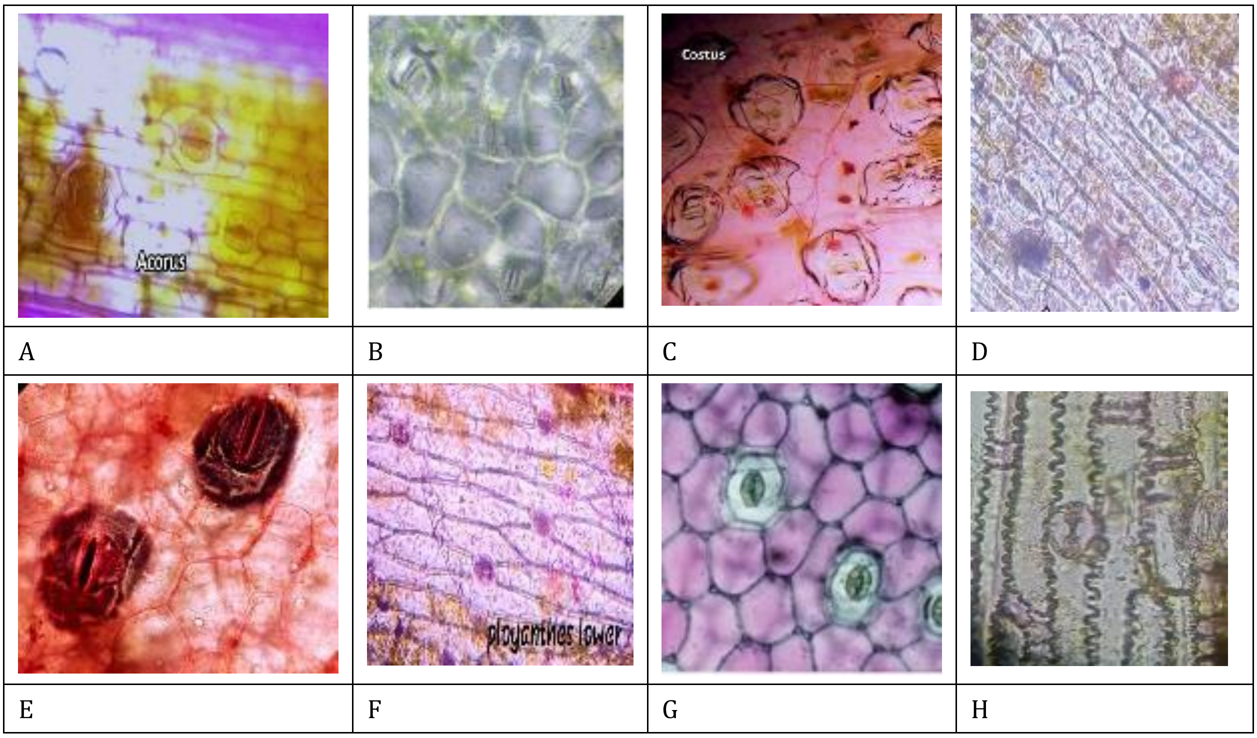

Figure 4 Diversity of monocot stomata

[(A) *Acorus calamus* (Acoraceae), Paracytic stoma with a pair of lateral subsidiary cells, with oblique cell walls, In this "paracytic-oblique" type, the lateral subsidiary cells are only slightly smaller than pavement cells and clearly belong to the adjacent cell file. (B) *Caladium bicolor* (Araceae) , Brachyparacytic stomata with oblique cell walls (C*) Costus speciosus* (Costaceae) Paracytic stomata with oblique cell walls (D) *Dracaena marginata* (Asparagaceae) Anomocytic stomata (lacking subsidiary cells) , (E) *Kaempferia galanga* (Zingiberaceae) , Tetracytic stomata (F) *Polianthes tuberosa* L. (Amaryllidaceae) , Anomocytic stomata, (G) *Tradescantia spathacea* (Commelinaceae) Tetracytic stomata, (H) *Cynodon dactylon* (L.) Pers.(Poaceae) paracytic stomata with dumbbell shaped subsidiary cells.]

3.4. Dicotyledons plant material

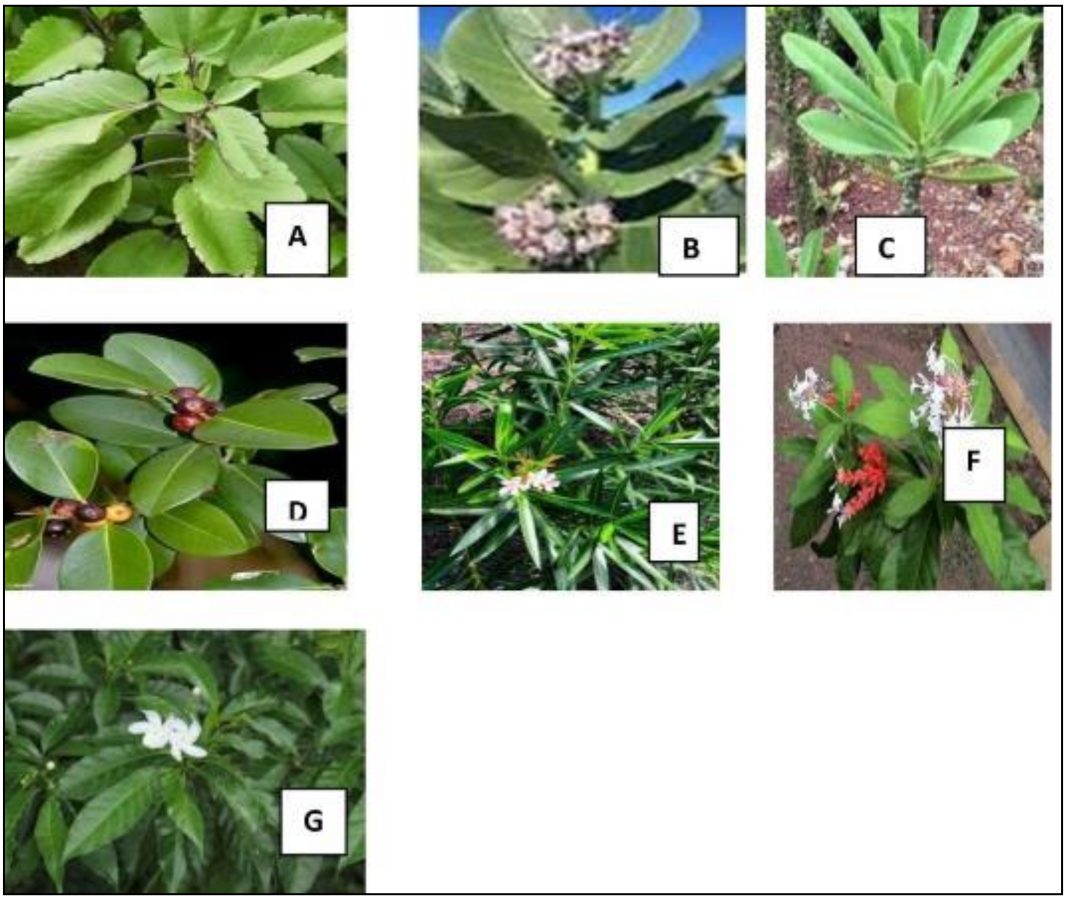

Figure 5 Dorsiventral Leaves in some dicots

[A. *Bryophyllum calycinum* (Crassulaceae) B. *Calotropis procera* (Asclepiadaceae), C. *Euphorbia neriifolia* (Euphorbiaceae), D. *Ficus microcarpa* (Moraceae), E. *Nerium oleander* (Apocynaceae), F. *Rauwolfia serpentina* (Apocynaceae), G. *Tabernaemontana divaricata* (Apocynaceae)]

Table 3 Dicot taxa studied and their stomatal features

No.	**Species**	**Family**	**Type**	**Density (stomata /mm^2) in lower surface**	**Index (%)**	**Adaptation**	**Group**
1	*Bryophyllum calycinum*	Crassulaceae	Anisocytic, Amphistomatic	50.54 ±1.2	11.11±2.3	Crassulacean Acid Metabolism (CAM), a physiological adaptation	Succulent xerophytes
2	*Calotropis procera*	Asclepiadaceae	Anomocytic, Amphistomatic	90.8±2.1	20±3.3	Xeric and semi-xeric	Non-succulent xerophytes
3	*Euphorbia neriifolia*	Euphorbiaceae	Anomocytic, Hypostomatic	22.7±1.0	5±2.2	During high temperatures or dry conditions,	Succulent xerophytes

						conserve water	
4	*Ficus microcarpa*	Moraceae	Anisocytic, Hypostomatic	20.29±1.22	4.47±1.2	Adaptable plant for the semi-arid and arid regions	Non-succulent xerophytes, hypostomatic
5	*Nerium oleander*	Apocynaceae	Anisocytic, Hypostomatic and sunken	48.35±2.2	10.651 ±2.6	Adaptable plant for the semi-arid and arid regions	Non-succulent xerophytes
6	*Rauwolfia serpentina*	Apocynaceae	Anomocytic, Hypostomatic	55.02±2.1	12.12±1.2	Adaptable plant survives in moderate climates	Mesophytic herb
7	*Tabernaemontana divaricata*	Apocynaceae	Anomocytic, Hypostomatic	59.83±3.3	13.8±2.6	Adaptable plant survives in moderate climates	Mesophytic shrub

3.5. Dicot taxa studied and their stomatal features

The Dicot taxa studied (Fig.5) along with their stomatal features (Fig. 6) were summarized in Table 3.

3.5.1. Family: Crassulaceae *(Bryophyllum calycinum)*

It was found that the plant exhibited anisocytic and amphistomatic stomata. The anisocytic stomatal type represented an intermediate evolutionary state, more advanced than anomocytic configurations but less specialized than tetracytic stomata. The combination of anisocytic stomata and CAM (Crassulacean Acid Metabolism) metabolism represented a dual adaptation strategy for maximizing water-use efficiency.

3.5.2. Family: Asclepiadaceae *(Calotropis procera)*

Stomata were irregularly scattered and sunken, amphistomatic, elliptical-shaped stomatal complexes. Epidermal pavement cells were rectangular, pentagonal, hexagonal, or octagonal in shape. The guard cells were surrounded by 4-5 subsidiary cells, which were not regular in shape and diacytic, paracytic, cyclocytic, anisocytic, or tetracytic type. Subsidiary cell arrangement of stomata had also been observed. The diversity in stomatal types and their arrangements demonstrateed evolutionary fine-tuning, allowing *Calotropis procera* to thrive in xeric and semi-xeric conditions.

3.5.3. Family: Euphorbiaceae *(Euphorbia neriifolia)*

The leaf of *E. neriifolia* showed anomocytic stomata surrounded with guard cells and 2–3 subsidiary cells. The adaxial side of the *E. neriifolia* leaf contained greater number of stomata than the abaxial side. The abaxial epidermis contained rectangular to circular shaped epidermal cells, and tubular to rectangular shaped adaxial epidermal cells. Xerophytes like *Euphorbia neriifolia* had anomocytic stomata to reduce water loss, making them better suited for dry environments.

3.5.4. Family: Moraceae *(Ficus microcarpa)*

Paradermal lower epidermis of the leaf of *Ficus* disclosed irregularly polygonal, slightly undulated and straight epidermal cells with straight walls, actinocytic stomata. Stomata hypostomatic between veins, sunken, cuticular thickening of stomata was well developed. The anatomical features highlighted the adaptability of plant for the semi-arid and arid regions.

3.5.5. Family: Apocynaceae *(Nerium oleander)*

The study of the plant revealed that the underside of the leaf contained stomata located at the base of small depressions known as stomatal crypts i.e., sunken stomata. These crypts, which were lined with tiny hairs, trap stagnant air and create a more humid microenvironment. As a result, when the leaf opened its stomata, water loss through transpiration was reduced compared to gas exchange occurring directly in the drier surrounding air. Anisocytic stomata were found within the small polygonal epidermal cells. Large numbers of epidermal hairs were found. These features contribute to efficient gas exchange and water conservation, underscoring the significance of stomatal evolution in plant survival and ecological success.

3.5.6. Family: Apocynaceae *(Rauwolfia serpentine)*

The leaf cotained anomocytic stomata and hexagonal shaped wavy walled epidermal cells. Stomata were only on the abaxial surface making it hypostomatous. Mesophytes like Rauwolfia serpentina, anomocytic stomata provide a balance between water conservation and efficient photosynthesis.

3.5.7. Family: Apocynaceae *(Tabernaemontana divaricate)*

It was found that anomocytic, hypostomatous condition and moderate stomatal density facilitated gas exchange while maintaining a balance in water loss.

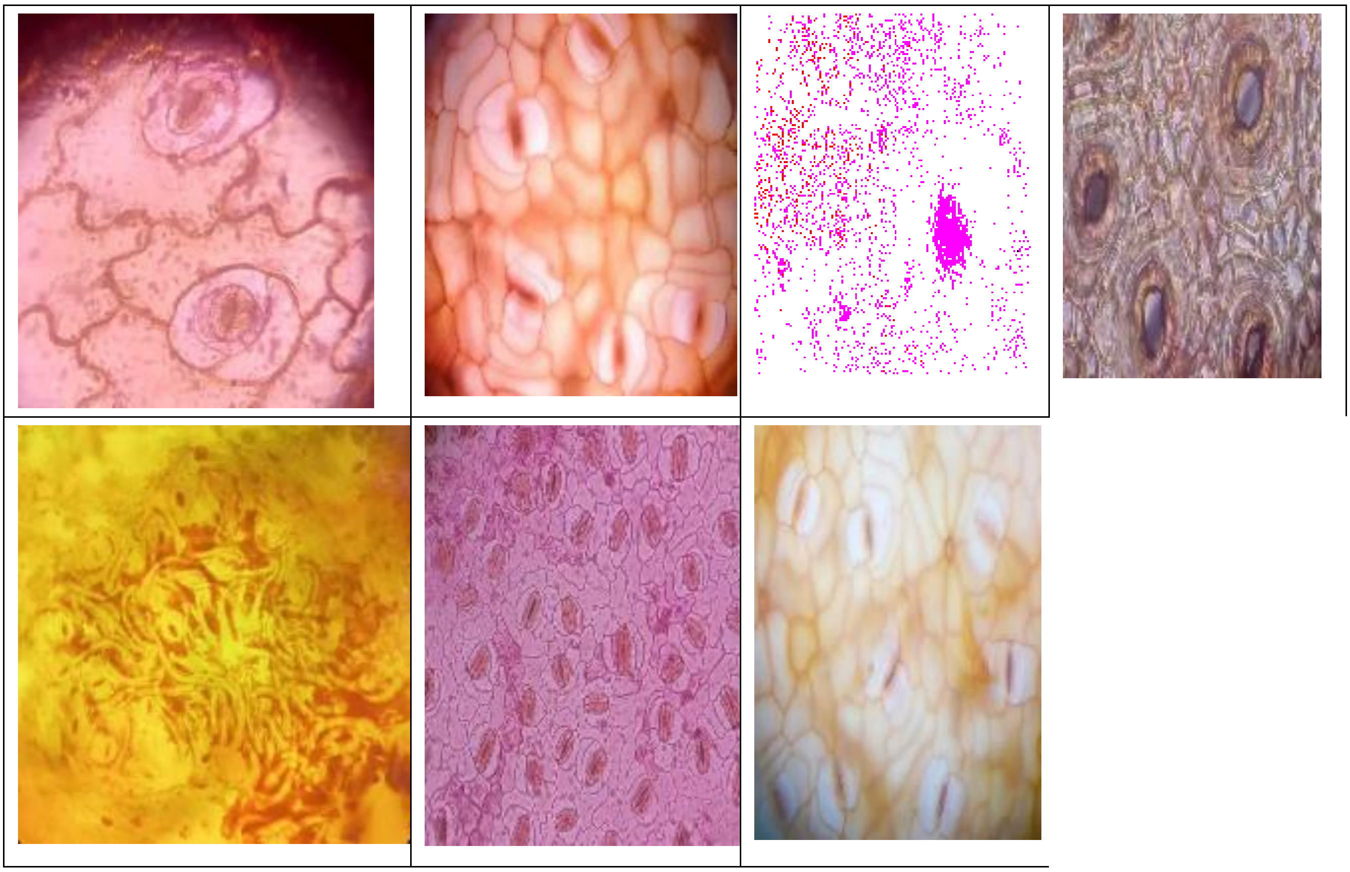

Figure 6 Types of dicot stomata

[A.*Bryophyllum calycinum* (Crassulaceae), Anisocyti stomata, B. *Calotropis procera* (Asclepiadaceae)) Paracytic stomata, C. *Euphorbia neriifolia* (Euphorbiaceae) Anomocytic stomata, D. *Ficus microcarpa* (Moraceae) Anisocytic stomata, E. *Nerium oleander* (Apocynaceae) Anisocytic stomata, F. *Rauwolfia serpentina* (Apocynaceae) Parasitic stomata, G. *Tabernaemontana divaricata* (Apocynaceae)Parasitic Stomata]

3.6. Diversity of stomata in the studied plants

The diversity of stomatal types observed in the studied plants reflects significant taxonomic and ecological variations. Stomatal morphology is an important adaptive trait that influences gas exchange, water regulation, and overall plant physiology [1, 16]. The present study reports a variety of stomatal types across the genera *Rauwolfia, Bryophyllum, Calotropis*, and *Tabernaemontana*, showcasing their structural diversity and potential functional implications (Fig. 7).

The presence of laterocytic stomata in *Rauwolfia* and *Calotropis* suggests a common structural adaptation in these genera. Laterocytic stomata, characterized by subsidiary cells positioned laterally, are known for their role in efficient water use and gas exchange [2]. Similarly, amphilaterocytic stomata found in *Bryophyllum* and *Rauwolfia* highlight a complex arrangement of subsidiary cells, which might contribute to their adaptability under varying environmental conditions [7]. *Bryophyllum* exhibits notable stomatal diversity, including amphilaterocytic, brachyparacytic, anisocytic, and diacytic types. The presence of anisocytic stomata, which have three unequally sized subsidiary cells, aligns with previous reports indicating their prevalence in drought-resistant species [17]. Additionally, diacytic stomata, with two subsidiary cells perpendicular to the guard cells, are known for their regulatory efficiency in water-stressed environments [16].

Tabernaemontana exhibits a wide range of stomatal types, including bipolaramphi brachyparacytic, incomplete brachyparacytic, stephanocytic, laterocytic, and brachyparahexacytic stomata. The occurrence of stephanocytic stomata, characterized by a ring of subsidiary cells surrounding the guard cells, suggests an evolutionary adaptation for optimized gas exchange [18]. The presence of incomplete brachyparacytic stomata may indicate intermediate evolutionary stages in stomatal differentiation.

The identification of unique stomatal types such as amphipsudoholoparacytic and incomplete amphipsudoholoparacytic in *Rauwolfia* suggests specialized physiological adaptations. These complex stomatal types may contribute to enhanced control over transpiration, particularly in fluctuating environmental conditions. The presence of cyclocytic stomata in *Calotropis,* characterized by multiple concentric subsidiary cells, has been associated with xerophytic adaptations that aid in water conservation [19].

Stomatal variation within and among species can be influenced by evolutionary pressures, ecological factors, and genetic constraints. The coexistence of multiple stomatal types within a single genus, such as Rauwolfia, suggests a degree of plasticity that may confer selective advantages in diverse habitats [7]. Additionally, these variations align with previous studies highlighting the role of stomatal architecture in plant-environment interactions [6].

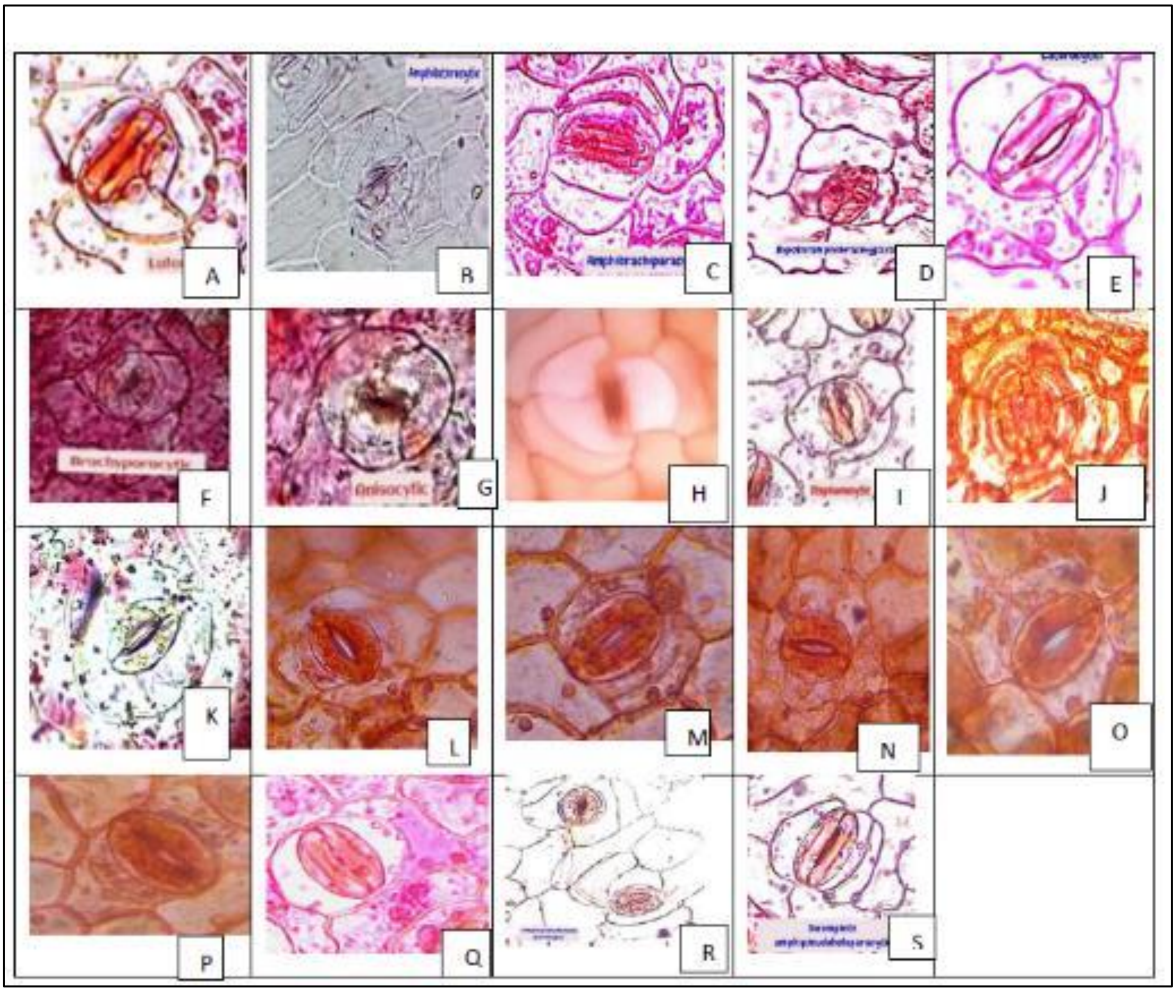

Figure 7 Diversity of stomata in the studied plants

[A.Laterocytic stomata(*Rauwolfia*), B.Amphilaterocytic stomata (*Bryophyllum*), C. Amphilaterocytic stomata(*Rauwolfia*), D. Bipolaramphilaterocytic stomata (*Rauwolfia*), E. Laterocytic stomata (*Rauwolfia)*, F.Brachyparacytic stomata (*Bryophyllum)*, G. Anisocytic stomata(*Bryophyllum*), H. Laterocytic stomata (*Calotropis*), I. Stephanocytic(*Rauwolfia*), J.Cyclocytic stomata(*Calotropis*), K.Diacytic stomata(*Bryophyllum*), L. Bipolaramphi brachyparacytic (*Tabernaemontana*), M. Incompltebrachyparacytic (*Tabernaemontana)*, N. Stephanocytic stomata *(Tabernaemontana*), O. Laterocytic stomata (*Tabernaemontana*), P.Brachy parahexacytic(*Tabernaemontana*), Q Brachyparacytic(*Rauwolfia*), R. Amphipsudoholoparacytic(*Rauwolfia)*, S. Incompleteamphipsudoholoparacytic (*Rauwolfia)*]

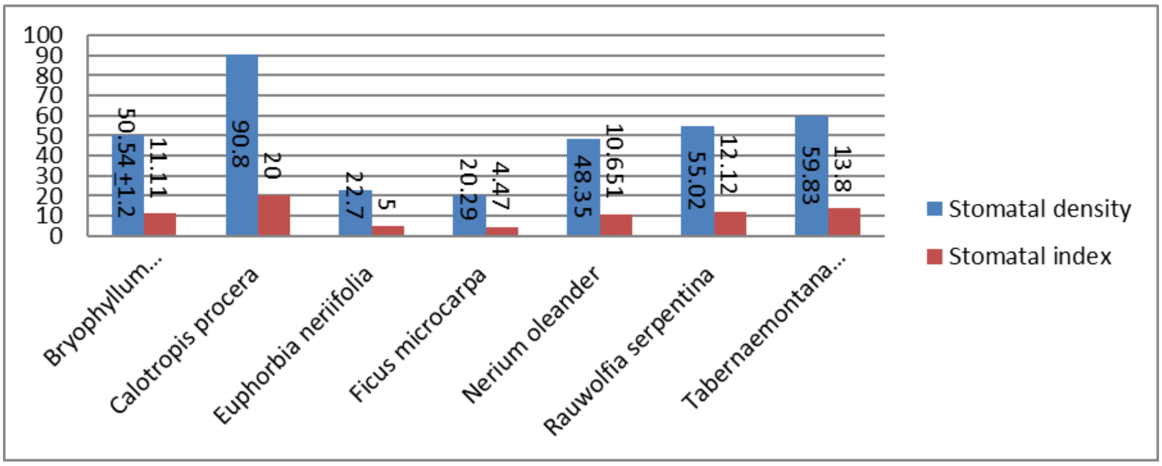

Figure 8 Dicot taxa studied and their stomatal features in graphical representation

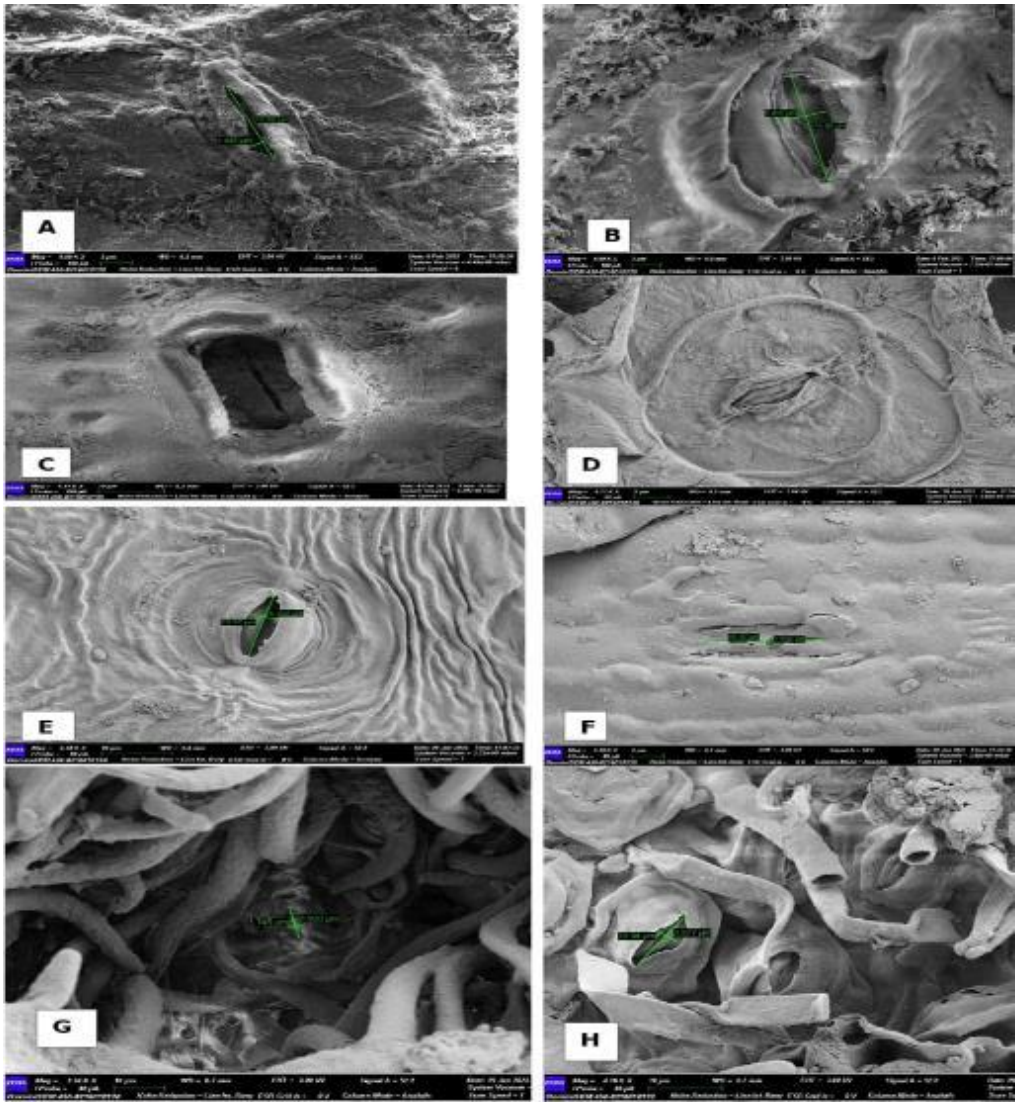

Figure 9 Photomicrographs (Scanning Electron Microscopy) of epidermal surfaces of few dicots and monocot. [A. *Caladium* X 6.60kX, B. *Euphorbia* X 7.52kX, C. *Polyanthes* X 4.17kX, D. *Bryophyllum* x 4.71kX, E. *Calotropis* X 4.34kX, F. *Dracaena* X 5.39Kx, G. *Nerium* x 2.74kX, H. *Nerium* X 4.16kX]

From the above results, it may be discussed that fifteen taxa distributed in 6 monocotyledonous families and 5 dicotyledonous families form the subject matter of the present discussion. Parasitic stomata observed common in *Calotropis procera* (Asclepiadaceae), *Rauwolfia serpentina* (Apocynaceae) and in *Tabernaemontana divaricata* (Apocynaceae) three plant species from different families. And anisocytic stomata observed common in *Bryophyllum calycinum* (Crassulaceae), *Ficus microcarpa* (Moraceae) and in *Nerium oleander* (Apocynaceae), This might indicate closeness or relationship of these families. While Anomocytic type of stomata found only in *Euphorbia neriifolia* (Euphorbiaceae) indicated their distinctness. It may be concluded from result that there can be presence of more than one kind of stomata observed in the family Apocynaceae.

The paracytic stomatotype was regarded as probably primitive in both dicotyledons and monocotyledons. Other types of stomata had repeatedly been derived from the paracytic type, so that the presence of similar advanced stomata types was not a sure indication of close relationship.

It was concluded that morphological classification of stomata is best based on their appearance in the mature leaf. Nonetheless, stomatal types and their ontogenies had been used with success in the resolution of many taxonomic problems. Stomatal density, index, and morphology were critical determinants of plant adaptation to diverse environments [3]. By analyzing these traits in the context of ecological grouping, it may be gained insights into the functional roles of different plant species and predict their responses to environmental changes [7]. Further research is needed to address existing research gaps and enhance our understanding of the genetic, developmental, and ecological mechanisms underlying stomatal adaptation [20]. This knowledge is essential for developing strategies to conserve plant biodiversity and manage ecosystems in a changing world [20]. The data showed a variety of mature stomatal types in monocots and dicots, classified as anomocytic, paracytic-nonoblique, or paracytic –oblique, and tetracytic. and anisocytic. Since both anomocytic and paracytic/tetracytic stomatal types were found in early-divergent angiosperms, the ancestral angiosperm condition remains uncertain [21,22]. Distinguishing monocots with broad leaves from dicotyledonous leaves can be challenging. For instance, fossil Smilax or *Dioscorea* (Monocot) are often difficult to differentiate from fossil Piperaceae (Dicot) [8].

Stomatal complexes serve as key diagnostic traits in plant taxonomy from a biosystematic perspective. Their stability under varying environmental conditions has led numerous studies to use them in distinguishing plant groups, resolving taxonomic controversies, and even interpreting phylogenetic relationships [21]. Additionally, the importance of foliar epidermal characteristics in plant taxonomy has been widely recognized across various plant groups [23]. Numerous researchers have highlighted the influence of stomatal and epidermal cell characteristics on plant drought resistance and water use efficiency. The response of these characteristics to drought conditions can differ among plant species and varieties. Many studies suggest that plant adaptation to drought stress may involve an increase in stomatal density and/or a reduction in stomatal size.

In this study, we examined the quantitative and qualitative characteristics of epidermal cells and stomata in leaf surface of 15 taxa from dicot and monocot. The epidermal cell and stomatal traits observed on leaf surfaces exhibit variation among different taxa This diversity in stomatal types has been previously documented in Fabaceae [1,23]. As most plants close their stomata in response to drought stress, subsidiary cells assist guard cells in this process by offering a mechanical advantage that enhances guard cell movement and by acting as a reservoir for ions and water. Results from our observations showed anisocytic stomata as the dominant stomatal complex in dicotyledonous plants, along with a few anomocytic stomata. In the studied of 8 monocotyledonous plants showed paracytic stomata as dominant, hemiparacytic, brachyparacytic, actinocytic, cyclocytic, and diacytic stomata, as well as five types of abnormal stomata, including adjacent stomata sharing a common subsidiary cell, giant stomata, juxtaposed stomata, stomata with only one guard cell, and superposed stomata. In the present study, some stomatal types, particularly abnormal ones, are newly recorded and described. Therefore, this research contributes to the micro-morphological understanding of the studied genera.

3.7. Abnormal stomata and guard cells

Stomatal abnormalities are significant indicators of developmental plasticity and environmental adaptability in plants. The presence of abnormal stomata and guard cells in both monocot and dicot species, as observed in the present study, provides insight into the diversity of stomatal development across different taxa(Fig. 10). These anomalies may be attributed to genetic regulation, environmental factors, or both [24].

The occurrence of non-contiguous stomata in *Tabernaemontana* and *Rauwolfia* suggests a deviation from the normal stomatal patterning. Non-contiguous stomata, where stomata are widely spaced apart, may arise due to irregular meristematic activity during epidermal development [16]. Such spatial distribution could influence transpiration rates and gas exchange efficiency, impacting the overall physiological responses of the plant.

Paired stomata, found in *Rauwolfia* and *Tabernaemontana*, indicate a rare developmental anomaly where two stomata are adjacent to each other without intervening epidermal cells. This condition is often associated with irregular cell division in stomatal precursors [25]. While such formations are not frequent, their presence suggests a possible mutation or environmental stress-induced developmental shift.

The presence of single guard cell stomata in *Tabernaemontana*, *Dracaena, Polianthes,* and *Rauwolfia* is an unusual phenomenon. Stomata typically functions with paired guard cells to regulate gas exchange. However, in certain species, single guard cell stomata can form, potentially affecting stomatal functionality under stress condition [26].

The identification of abnormal guard cells in *Calotropis* highlights disturbances in normal stomatal ontogeny. Abnormal guard cells may arise from incomplete differentiation or mechanical constraints during cell expansion [1]. Such irregularities may exhibit the efficiency of stomatal opening and closing, thereby affecting transpiration dynamics.

Arrested stomata, found in *Calotropis*, represent stomatal structures that have failed to mature fully. This developmental stagnation was found from genetic mutations, hormonal imbalances, or abiotic stress factors [27]. Arrested stomata are often non-functional, leading to reduced stomatal conductance and possible physiological impacts for the plant.

The existence of stomata with large meristemoids in *Bryophyllum* suggests prolonged meristematic activity before guard cell differentiation. This could indicate a prolonged developmental phase or an anomaly in the regulatory mechanisms which controls meristem size [28]. Larger meristemoids might contribute to increased stomatal production under favourable conditions is the reflection of developmental lag in epidermal cell division.

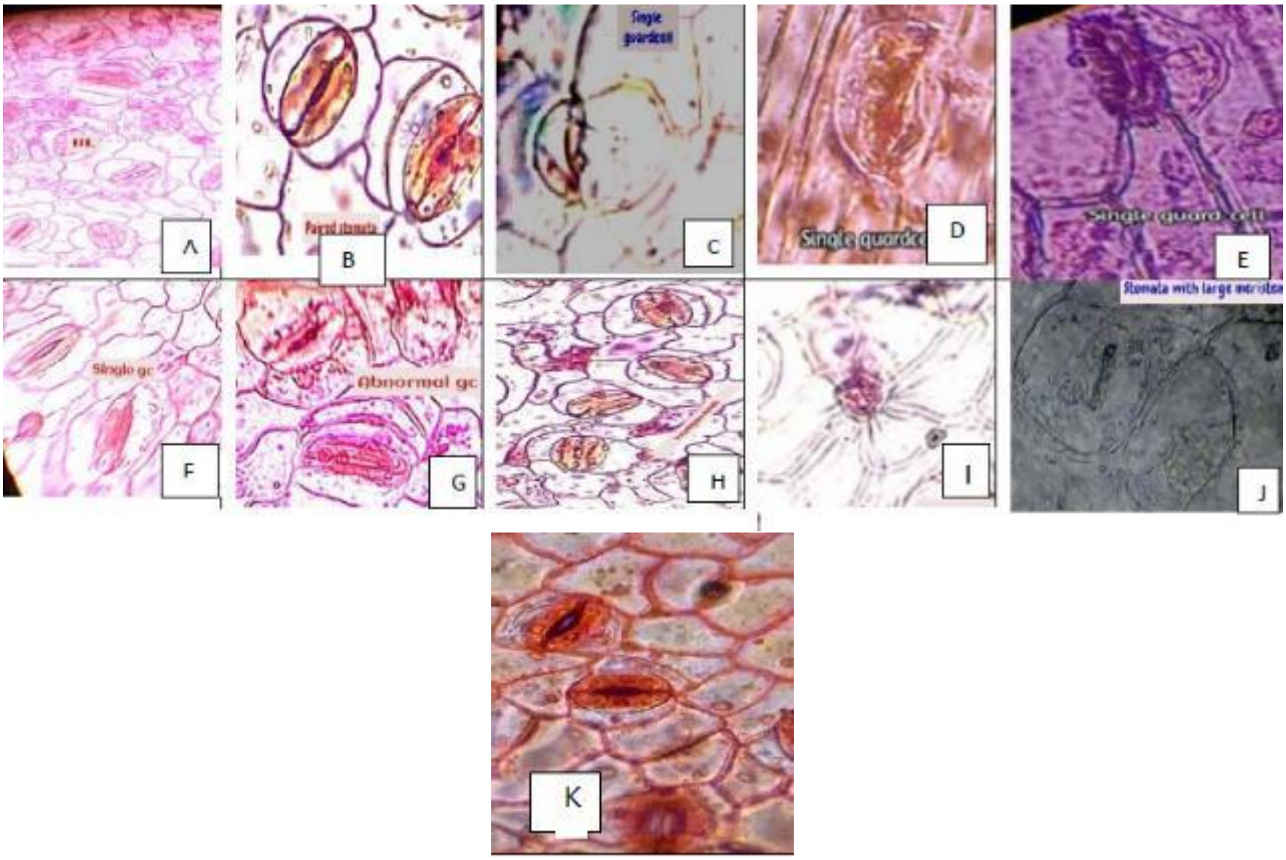

Figure 10 Abnormal stomata and guard cells of leaves of studied species of the monocots and dicots

[A.Non-contiguous stomata in *Tabernaemontana,* B. Paired stomata in *Rauwolfia* C.Single guardcell in *Tabernaemontana*, D. Single guardcell in *Dracaena*, E. Single guardcell in *Polyanthes*, F. Single guardcell in *Rauwolfia*, G.Abnormal guardcell in *Calotropis*, H. Non-contiguous stomata in *Rauwolfia*, I. Arrested stomata in *Calotropis*, J. Stomata with large meristemoid in *Bryophyllum*, K. Paired stomata in *Tabernaemontana]*

4. Conclusion

The diversity of stomatal types across monocot families reflects evolutionary adaptations to varied ecological conditions. The presence of brachyparacytic stomata in *Acorus calamus* represents an evolutionarily primitive trait, whereas the tetracytic condition in *Kaempferia galanga* and *Tradescantia spathacea* suggests advanced regulatory mechanisms for efficient gas exchange. Hypostomatic species like *Costus speciosus* and *Tradescantia spathacea* indicate specialization towards water-use efficiency, while amphistomatic taxa (*Acorus calamus, Caladium bicolour, Dracaena marginata, Kaempferia galanga*) exhibit traits favouring humid environments. The anomocytic stomata in *Dracaena marginata* and *Polianthes tuberosa* link these taxa to basal monocot lineages, underscoring their primitive yet functional adaptations. Meanwhile, *Cynodon dactylon* exemplifies the unique stomatal morphology of Poaceae, with dumbbell-shaped guard cells and dome-like subsidiary cells, optimizing transpiration control in grasses. These variations highlight the intricate evolutionary pathways of monocots, demonstrating how stomatal structure has played a crucial role in their success across different habitats. On the other hand, the presence of anisocytic stomata in dicot plants like *Bryophyllum calycinum*, coupled with CAM metabolism, represents a dual strategy for optimizing water-use efficiency in arid environments. Similarly, *Calotropis procera* exhibits a remarkable diversity of stomatal types, with irregularly scattered and sunken stomata, suggesting fine-tuned physiological mechanisms for survival in xeric and semi-xeric habitats. Sunken stomata in *Nerium oleander* and *Ficus microcarpa*, often accompanied by cuticular thickening and stomatal crypts, further enhance drought tolerance by reducing transpiration. Overall, the diversity in stomatal types, subsidiary cell arrangements, and stomatal distribution patterns among these dicot species highlights significant evolutionary advancements toward ecological specialization. Detailed investigation of both adaxial and abaxial epidermal surfaces under light microscope revealed five major stomatal types and eleven subtypes. Scanning electron microscopy (SEM) revealed that stomata were sunken in the epidermis. The diverse array of stomatal abnormalities observed in the present study underlines the complexity of stomatal development and variations across different species suggest an interplay of genetic and environmental factors influencing epidermal differentiation. Thus, stomatal traits in monocot and dicots influenced a great in adopting different environmental variations with different ecological classification.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest.

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