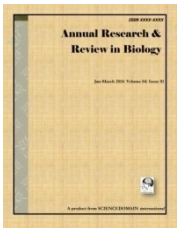

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Stomatal Patterning: An Important Taxonomic Tool for Systematical Studies of Tree Species of Angiosperm

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Authors' contributions

This work was carried out in collaboration between all authors. Author FK designed the study, standardized the protocol and wrote the manuscript. Author ZY over all supervision and performed the statistical analysis. Authors AA, HAR, AY and MR managed the literature searches. Authors HSA, ZT and NR managed the analyses of the study. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Taxonomic information provides the base line for most of the studies in biological sciences. Most of taxonomic information based on phenotypic characteristics of plants. As phenotypic markers are under the influence of environment, therefore it may leads toward the taxonomic confusion. Therefore, present study was carried out to determine the effect of environment on types of stomata, number, size, and stomata patterning which is very useful feature in taxonomy. In the present study thirty arboreal species of dicot flora (from tropical and subtropical regions) belonging to eight orders and fifteen families are evaluated by using systematic tool i.e. stomatal pattering. Stomata play a vital role in gas exchange of dicot plants. Within dicot flora, eight shapes of stomata are observed (anomocytic, anomotetracytic, actinocytic, amphianisocytic, brachyparacytic, anisocytic, amphicyclocytic and staurocytic). In leaves, the pattern of stomatal distribution is highly variable between arboreal species of dicot but is regulated by a mechanism of one cell spacing between stomata. Epidermal anatomical marker showed the different mode of

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stomata patterning. Hence, this differential marker can be utilized to differentiate taxonomically complicated species.

Keywords: Anomocytic; anomotetracytic; actinocytic; amphianisocytic; brachyparacytic; anisocytic; amphicyclocytic; staurocytic; stomatal pattern.

1. INTRODUCTION

The orderly placement of stomata on leaf surface is called stomata patterning. Stomatal patterning is process, which involves selection of undifferentiated cell to become stomata. There is variety of mechanism contributed to stomata patterning [1]. Functioning of stomata is directly related to its distribution pattern. All the different division of Plants have different pattern of stomatal distribution [2]. The distribution pattern is not only under the control of genetics, but also foremost environmental factors. Such as light intensity, humidity, temperature, atmospheric carbon dioxide and nutrient availability and by the internal architecture and inserted level of leaves [3]. An extensive list of factors affecting the stomatal properties and their distribution on the leaves has been compiled by [4]. In dicots, the stomata are usually scattered on leaf epidermis and separated from one another by a stomata-free region, which inhibit direct contact between the stomata [5,6]. This stomatal pattern is important not only for the optimal functioning of stomata but also for ecological and evolutionary significance.

Stomatal development in dicots begins with an asymmetric cell division in which the smaller of two daughter cells directly rise to guard cell. It is clear demonstration of nonrandom arrangement of stomata in the leaf epidermis, proposed by [7,8]. Mostly, the stomatal patterning in vascular plants effected by oriented asymmetric and symmetric cell division [7,8]. Therefore in some dicot species, the stomata are scattered in free-distributed regions while in some species the stomata are arranged in clustering orientation [9]. Cluster stomata is a group of two or more stomata that indicate after forming the stomatal chamber in which the individual stomata are arranged separately from one another by subsidiary cells [10]. Stomatal cluster have been observed in 38 genera of 19 vascular plant families, including Begonia (*Begoniaceaea*), Ficus (*Moraceae*), *Stachytarpheta* (*Verbenaceae*) and Sedum (*Rassuliaceaae*) [10,11,12]. However, these studies focused on the developmental mechanism and distribution pattern of the stomatal cluster but they have not use information for phylogenetic relationship of species.

They recorded that ratio of singly occurring stomata to stomatal units is greater in younger leaves than in mature leaves .The number of stomata in a stomatal cluster varies. It was verified that in plant species which have only singly occurring stomata, the stomata will be scattered than the grouped on the leaf surface in the early stage of development [13]. Unfortunately, Most of the taxonomic information of plants accumulated so far is based solely upon morphometry.

As most of the taxonomist were agreed that similarities and dissimilarities of plants could measure by using morphological markers [14]. Although morphological characters are directly exposed to environment and can cause change in morphology and leads toward the taxonomic confusions. Morphometry could not provide solution of complex taxonomic problems. Therefore taxonomist involved other biological techniques like leaf epidermal anatomy, cytology, electrophoresis as taxonomic tools [14]. The main objective of present

study to evaluate affectivity of stomata pattern as an important taxonomic tool for the systematical studies of arboreal species.

2. MATERIALS AND METHODS

Present study was conducted in Molecular Taxonomy Lab of Department of Botany, Lahore College for Women University, Jail Road Lahore, Pakistan. The experiments were performed during August 2009-August 2010.

2.1 Plant Material

For leaf, epidermal anatomical studies fresh leaves from living specimens were used. Fresh materials of different arboreal species were collected from the different localities of Pakistan and to have complete range of tropical and subtropical arboreal species under various conditions.

2.2 Isolation of Leaf Epidermis

For isolation of leaf adaxial and abaxial epidermis leaves were soaked for 3-4 days. Time of soaking was varied according to the texture of leaves. Epidermal samples were prepared according to the modified method of [8], who followed [15] technique. The fresh leaves were placed in test tube filled with 88% lactic acid kept in hot boiling water for about 3-4 hours. Lactic acid is used to soften the tissue of leaf due to which its peeling off is made possible.

2.3 Preparation of Slides

A sharp blade is used for peeling of leaf material. The epidermis was cut across the leaf and scrapped away together with the mesophyll cells until only the epidermal layer of the leaf remained on the slides. Both abaxial and adaxial sides of leaves were prepared and observed under the light microscope (model: Meiji techno).

2.4 Sampling and Scoring

To describe the stomatal distribution pattern three young leaves before development of guard cell and three mature leaves in which guard cells are properly developed guard cells were collected .The average area of the young leaf is 25.5 cm^2 and that of mature leaf is 58.95 cm^2.Epidermal samples were prepared according to the modified method of [16] who followed [15] techniques. 2mm × 2mm area are taken from the replica at intervals of 2 or 3mm and theses were studied by light microscope .Each stomata cluster or singly occurring stomata was scored as stomatal unit (Su) [17]. Derived the three indices as Stomatal unit density (SuD), stomatal unit size (SuS) and stomatal density SD. The SuD was calculated as the number of super square millimeter of the leaf surface. The data was represented in three dimension certain co-ordination system. The x and y represent the special location of the midpoint of the sampling square. Thex, y, z co-ordinate so formed analyzed using GS software (Gemma software design). Su, SuD and SD for each of the leaves were subjected to correlation analysis using SPSS 10.0 for Windows.

2.5 Photographs of Slides

Microphotographs were taken by using CCD digital camera (model: canon Pc1200 attached with MD lens MA151/30/73opter) fitted on light microscope (model: meiji techno). Identification of anatomical character was made by using power at high power plan (40×/0.65,∞/0.17, F=200, WD=0.5) and at lower power plan is (10×/0.25,∞/0.17, F=200, WD=7.3). These micrographs were useful for identification and differentiation of epidermal cells on the basis of microscopic features.

The stomatal index (SI) and guard cell areas (GA) were calculated as per [18,19] respectively:

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

Where S=number of stomata per unit area of ×10 objective of light microscope E=number of epidermal cells in the same unit area above.

2.6 Statistical Analysis

Data was evaluated by calculating variation of stomata index. The terminology used in describing stomatal types is that of [20].

3. RESULTS AND DISCUSSION

The main purpose of this study was to investigate the significance of stomatal patterning for the systematical studies of some arboreal species collected from tropical and subtropical regions. Different types of stomata were studied by [21], followed by [22], they recognized four broad categories of stomata based on the presence and arrangement of accessory cells as well as their mode of development. Stomatal patterning is related to ordered placement of stomata on the leaf surface. The pattering process involved the selection of undifferentiated cells to become stomata [22]. However, it has not any concerned with the physical events of their differentiation.

3.1 Stomatal Types

In dicotyledonous arboreal species, stomata patterning classified that based on shapes and arrangement of subsidiary cells and distribution pattern. In present study eight different types of stomata were recognized. These are as 1. Anomocytic, 2. Amphianisocyic, 3. Brachyparacytic, 4. Anomotetracytic, 5. Anisocytic, 6. Amphicyclocytic, 7. Actinocytic and 8. Staurocytic (Fig. 1). Schemes of stomatal typology [23,24] are based on the presence or absence of subsidiary cells relative to guard cells, and the ancestral origins of cells within the stomatal complexes. Based on arrangement the guard epidermal cell neighboring the guard cell more than 25 main types of stomata in dicot have been recognized [9]. Stomata surrounded by subsidiary cells that are somewhat radically elongated were identified as actinocytic. This modified form [14] were found in family Myrtaceae. This family is economically important as it contain tree species like *Sygzgium aromarticum* L. These species have irregular, wavy double layered epidermal cells. Actinocytic stomata were present on adaxial surface (Fig. 2). However, the number of subsidiary cells varies from four to five.

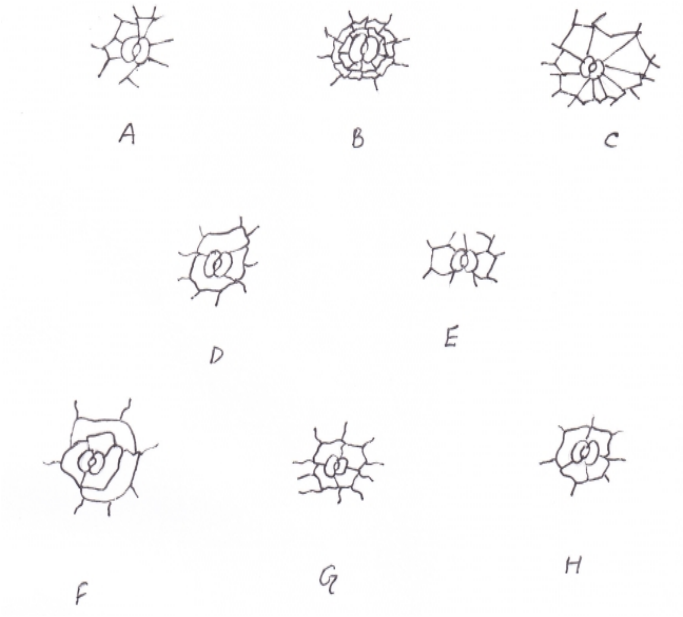

Fig. 1. Different Shapes of Stomata, A: Anomocytic, B: Amphicyclocyic, C: Actinocytic, D: Anisocytic, E: Barachy paracytic, F: Amphianisocytic, G: Staurocytic, H: Anomotetracytic

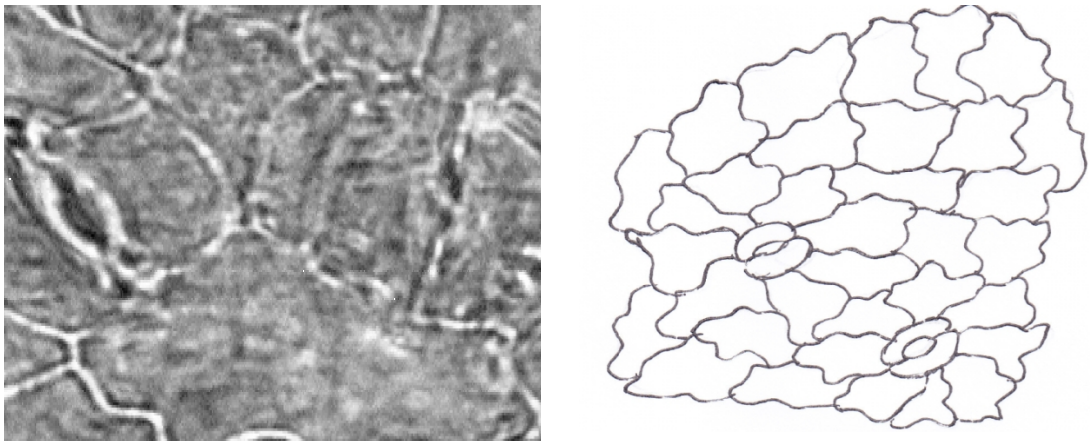

Fig. 2. Actinocytic type of stomata is shown in *Sygzgium aromarticum* L

3.2 Anomocytic

Epidermal cells around the guard cells not distinguishable from other epidermal cells. Family listed as *Myrtaceae*, *Malvaceae*, *Magnoliaceaea* and *Lythraceae*. *Azadirachta indica* L. (family *Meliaceae*) has pentagonal and smooth with double layered. Anomocytic stomata are present on both surfaces (Fig. 3). Number of stomata /unit area is 4-3. Number of subsidiary cells is seven. Smooth and polygonal having doubled layered found in *Callistemon lanceolatus*, D. C. Number of anomocytic stomata /unit area are 6-12 on both surfaces.

Double layered with smooth and polygonal epidermal cells are present in *Ficus infectoria* Roxb. Sana (family *Moraceae*).Number of stomata /unit area is 2-3.Development of anomocytic stomata are absent in abaxial surface (Fig. 4). In *Ficus racemosa* (family *Moraceae*), epidermal cells are arranged as polygonal and smooth with double layered. Anomocytic stomata are absent on abaxial surface.

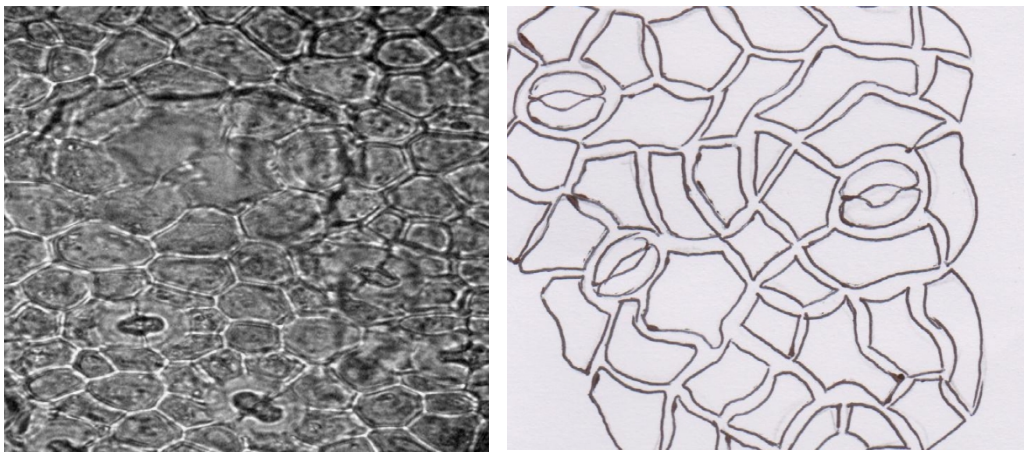

Fig. 3. Anomocytic type of stomata is shown in *Azadirachta indica* L

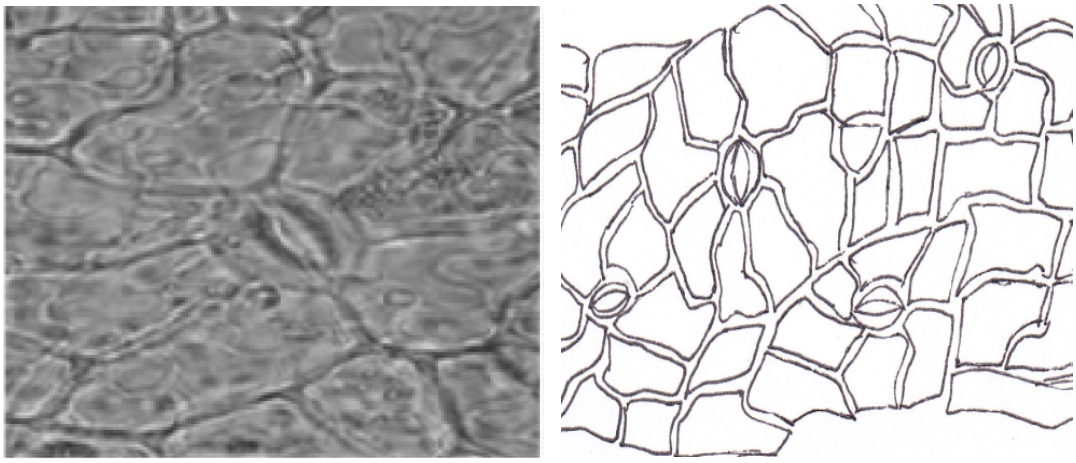

Fig. 4. Developmental stomata and patterning of *Ficus infectoria* and *ficus racsmosa*

3.3 Anisocytic

Stomata are surrounded by three cells, one of which is usually smaller than the other two, included families *Apocynaceae*, *Bignoniaceae*, *Leguminoseae*, *Lythraceae* and *Moraceae*. Epidermal cells are arranged as smooth and rectangular with single layered in *Murraya koenigi* L. Anisocytic stomata are found on both surfaces. Numbers of subsidiary cell is three. Number of stomata /unit area is 5-10. In *Acacia arabica*, Stewart; rectangular cells are arranged with single membrane and development of stomata on both surfaces. Number of stomata is three and Number of stomata/unit area is 5-10 on both surfaces.

Alstonia scholaris have smooth and pentagonal epidermal cells with double layer on both sides (Fig. 5). But Anisocytic stomata are present on adaxial surface. Number of subsidiary cells is three. Number of stomata /unit area is 1-2. This type of stomata are absent from abaxial surface. Epidermal cells are arranged as hexagonal and smooth on both abaxial and adaxial surfaces in *Artocarpus integifolia*. Anisocytic stomata are present only on adaxial surface but absent in abaxial surface (Fig. 6). Whereas Smooth, hexagonal with single layered cells and anisocytic stomata are present *Cassia fistula* Linn. In this specie, stomata are going to develop in some slides while some where it is examined the clustering of stomata on both surfaces. Numbers of stomata/unit area is 3-4. Numbers of subsidiary cells is five (Fig. 7). According to epidermal morphology and structure, polygonal, smooth and single layer cells are present in *Dalbergia sisso*, Roxb. Although this species is economically important. Due to highly absorption of CO2, patterning of anisocytic stomata are observed on both surfaces (Fig. 8). Numbers of stomata /unit area is 2-3. Number of subsidiary cell is three.

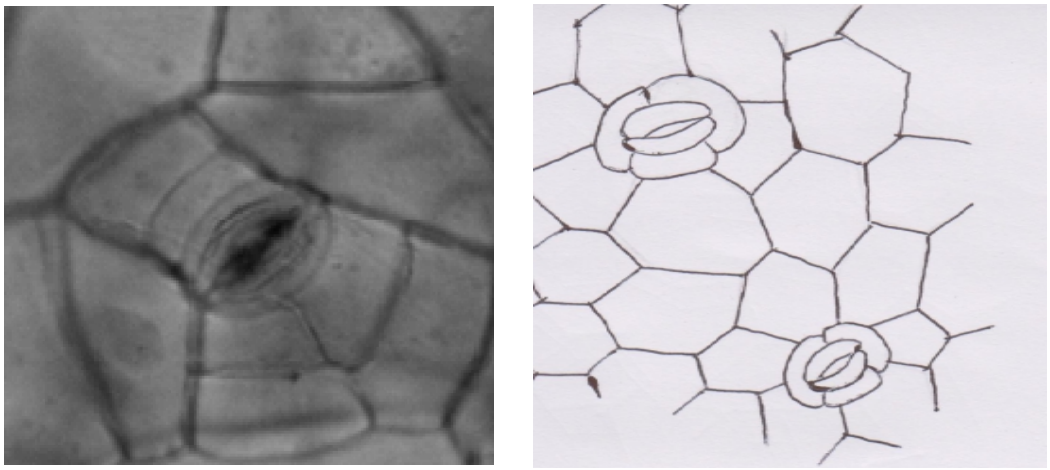

Fig. 5. Developmental stomata: And stomatal pattering in *Alstonia sacholaris*

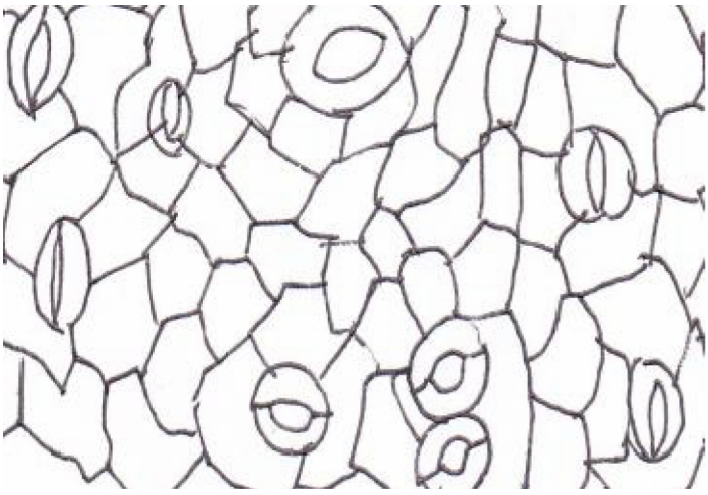

Fig. 6. Stomatal patterning in *Artocarpus integifolia*

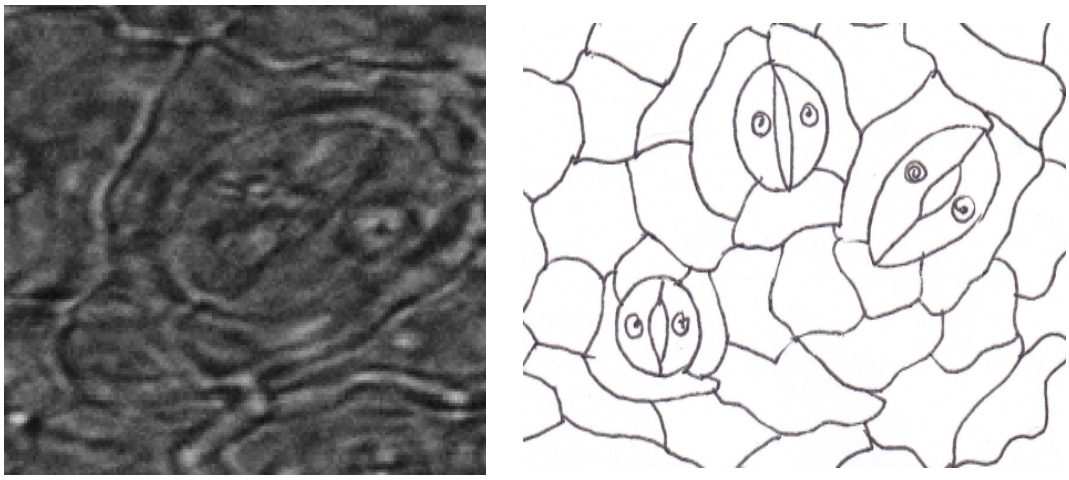

Fig. 7. Developmental stomata and stomatal pattering in *cassia fistula*

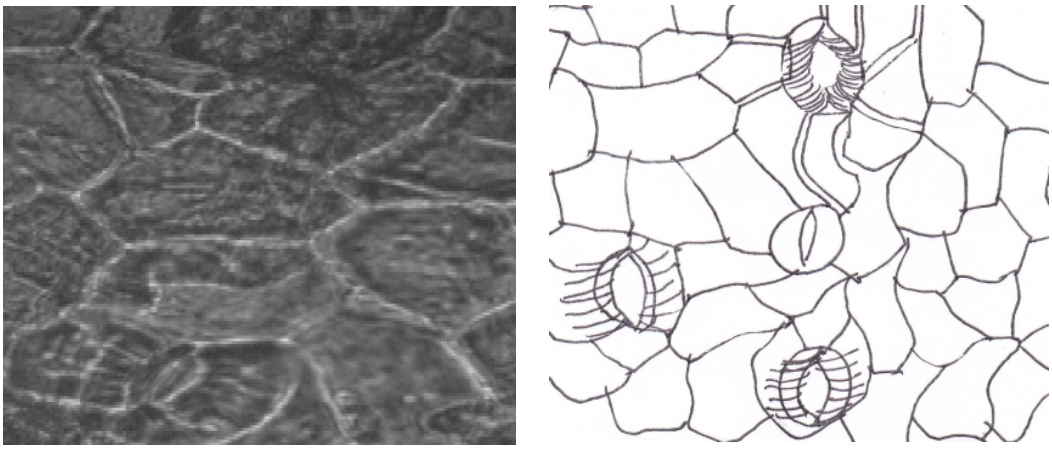

Fig. 8. Stomatal development and stomatal pattering (clustering) in *Dalbergia sissoo*

3.4 Brachyparacytic Stomata

Two cells flanking the sides of the guard cells but not completely enclosing them. This type of stomata is present in family *Euphorbiacaea*. Subsidiary cells may or may not elongated parallel to the long axis of the guard cells [24]. In *Albezia lebbeck* L (bent)., cells are arranged as irregular and wavy with double membrane on both adaxial and abaxial surface. Brachyparacytic stomata are present on both sides (Fig. 9). Numbers of subsidiary cells is five. While it is observed that epidermal cells are arranged as rectangular and smooth with double layered and brachyparacytic stomata on both abaxial and adaxial surfaces in *Eucalyptus camaldulensis* Dehnh (Fig. 10). Number of stomata /unit area is 2-4.Number of stomata is five. In *Averthoa carambola*, hexagonal and smooth cells are arranged with double layered and brachyparacytic stomata are present on both sides. Number of stomata /unit area is 5-9. Number of subsidiary cells is seven. The type of epidermal cells is arranged as pentagonal and smooth with double layered in *Lagerstroemia indica*. Number of stomata/unit area is 1-2. These types of brachyparacytic stomata are observed on adaxial

surface and absent on abaxial surface (Fig. 11). Similarly in *Putranjiva roxburji,* epidermal cells are arranged as pentagonal and smooth with double layered on both abaxial and adaxial surface. During all phase of growth and development, especially number of stomata is increased. It revealed the stomatal patterning on both sides (Fig. 12). Number of stomata is 1-2.Number of subsidiary cells is five. In *Saraca asoca,* an irregular and wavy cell with double layered are present on both sides. Brachyparacytic stomata are present only on adaxial surface (Fig. 13). Numbers of subsidiary cells is five. Number of stomata/unit area is 2-3.

Fig. 9. Developed stomata in *Albezia lebbeck*

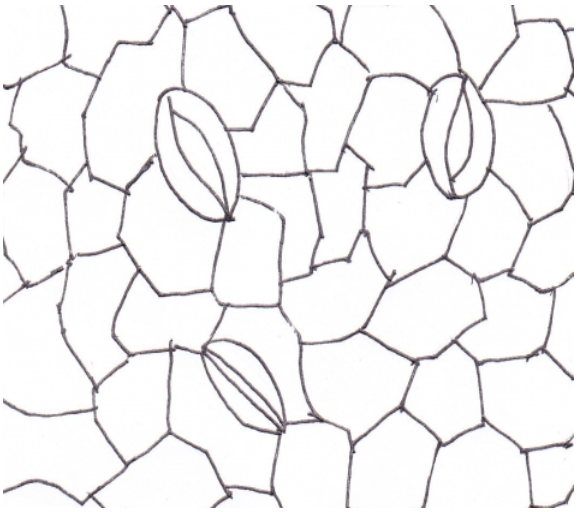

Fig. 10. Developed stomata in *Eucalyptus camaldulensis*

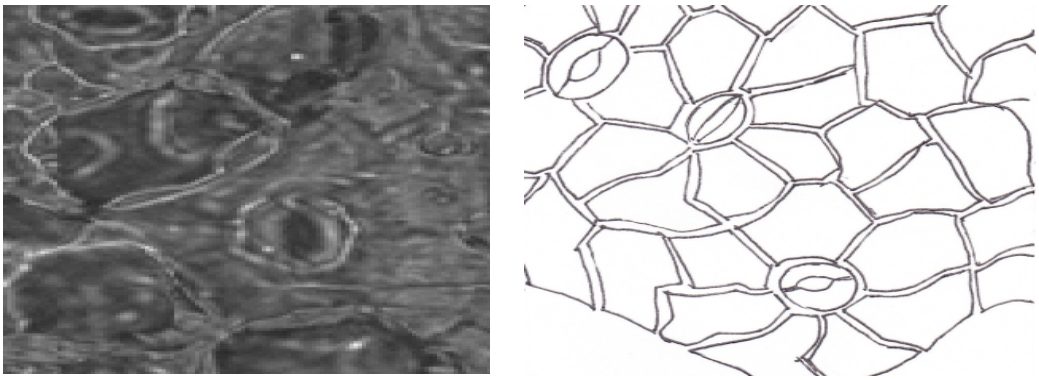

Fig. 11. Stomatal development and stomatal arrangement in *Lagerstromia indica*

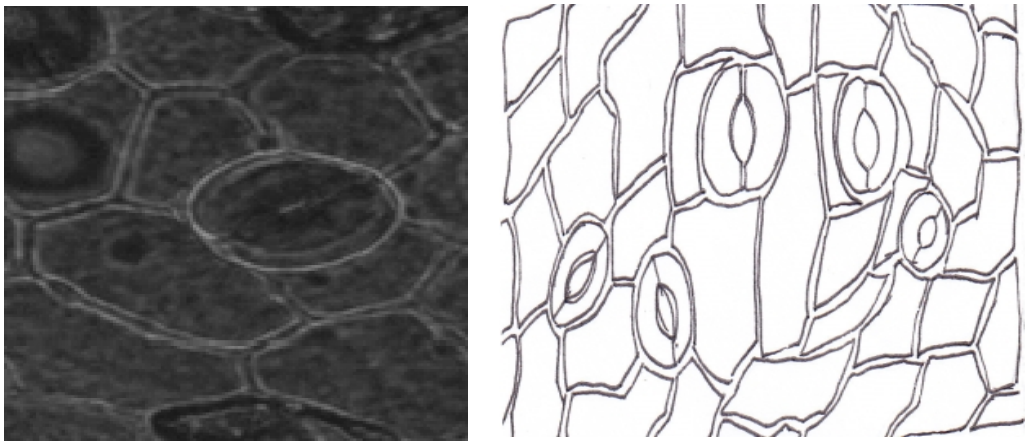

Fig. 12. Stomatal development and stomatal patterning in *Putranjiva roxburji*

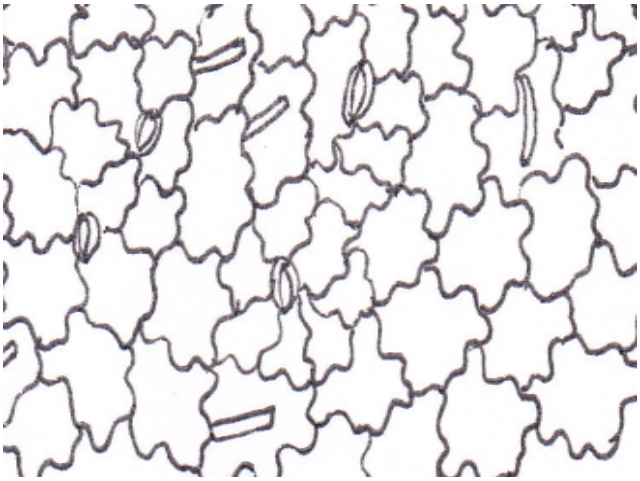

Fig. 13. Developed stomata and stomatal pattering in *Saraca asoca*

3.5 Staurocytic Stomata

Stomata surrounded by three to five similar subsidiary cells with anticlinal walls arranged cross-wise to the guard cells. In *Eryhrina subrosa,* polygonal and smooth with single layered. Staurocytic stomata are present only on adaxialsurface. Number of stomata is four. Smooth (Fig. 18) and hexagonal with single layer is showing staurocytic patterning of stomata on both surfaces in *Ficus religiosa*. Number of stomata /unit area is 4-6.Nnumber of subsidiary cells is four. Numbers of subsidiary cells is four.

3.6 Amphianisocytics Stomata

The Greek prefix amp, meaning around, double or on both sides is sometimes applied to leaves and stomata. For example: leaves are said to be amphistomata when the stomata are present on both surface. Wavy and irregular with double layered cells are observed in *Pongamia glabra*. Amphianisocytic stomata are present on both surfaces. Numbers of stomata /unit area is 2-3.Nmuber of subsidiary cells is five.

3.7 Anomotetracytic Stomata

Epidermal cells around the guard cells not distinguishable from other epidermal cells. Stomata are surrounded by four subsidiary cells, two of them parallel to the guard cells, the remaining pair being polar and often smaller .One polar cells is, or both are same times replaced by a single or a pair of ordinary epidermal cells, and this may happen at either pole or at both poles of the stomata. This term is defined by modification definitions given by [2]. *Melia azadirachta* have rectangular and smooth with double layered and anomotetracytic stomata are present on both surfaces. Number of stomata cells is four (Fig. 14). Anomotetracytic stomata, irregular and smooth double layered on both surfaces is examined in *Syzgium cumini*. Number of stomata /unit area is 30-40.Number of subsidiary cells is four. *Ficus glomerata* have polygonal and smooth double layered epidermal cells. In this species anomotetracytic stomata are observed only on abaxial surface. Number of stomata /unit area is 4-8.Number of subsidiary cells isfour. *Kigelia pinnate* have smooth and rectangular with double layered epidermal cells. Different phase of development of anomotetracytic stomata are found on both surfaces (Fig. 15). Number of stomata /unit is 5-6. Here polygonal, smooth with single layered cells are arranged in *Magifera indica*. According to growth phase of this species, the patterning of stomata are observed (Fig. 16). Whereas in *Magnolia grandiflora*, epidermal cells are wavy, polygonal of single membrane and anomotetacytic stomata are also present on both surfaces. Numbers of stomata/unit area is 3-4. Hence epidermal cells are arranged as polygonal and smooth having double membranes in *Sterculiaalata*. Anomotetracytic stomata are present only on adaxial surface, but absent on abaxial surface. Numbers of subsidiary cells is four. On the basis of cell arrangement, irregular and wavy epidermal cells are arranged in *Thevetia peruviana* with double membrane. At early stage of growth, anomotetracytic stomata are going to develop only on abaxial surface. Numbers of stomata /unit area is 5-6.Similarly in *Murraya paniculata*, epidermal cells are arranged as irregular and wavy having double membrane on both surface and *Anomotetracytic stomata* is observed on both surfaces. Number of stomata /unit area is 8-10. Number of subsidiary cells is four.

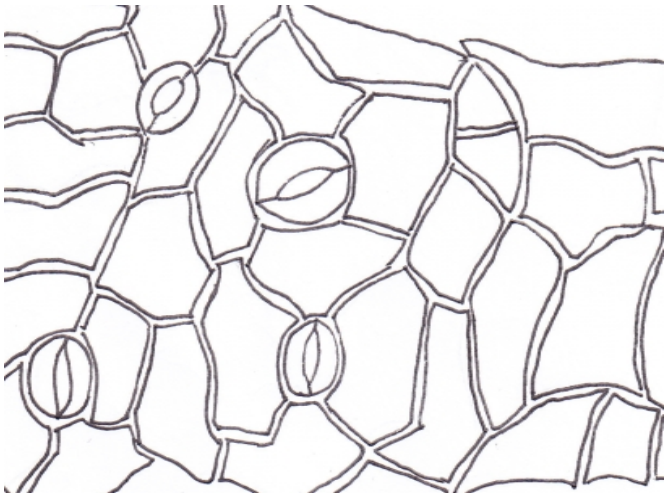

Fig. 14. Developed stomata *Melia azadirachta*

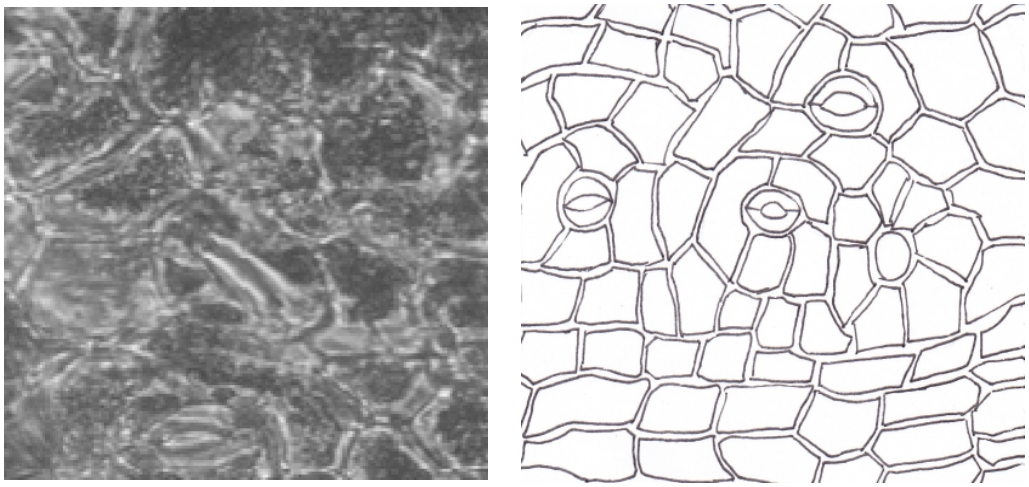

Fig. 15. Developed stomata and stomatal pattering in *Kigelia pinnata*

3.8 Amphicyclocytic Stomata

The Greek prefix amp, meaning around, double or on both sides is sometimes applied to leaves and stomata. So leaves are said to be amphistomata. Where the absence of patterning may indicate that such kind of species is less sensitive to CO2. According to epidermis morphology, irregular, wavy and double-layer cells are arranged in *Trewia nudiflora* on both surfaces. It is observed that stomata are going to develop at early stage. Numbers of subsidiary cells is three (Fig. 17).

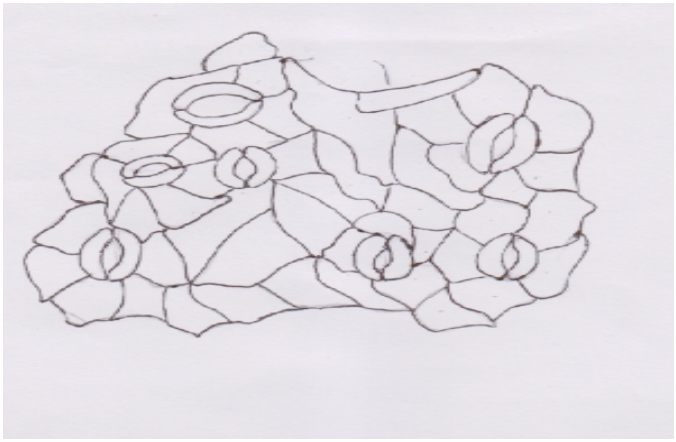

Fig. 16. Developed stomata and stomatal Patterning in *Mangifera indica*

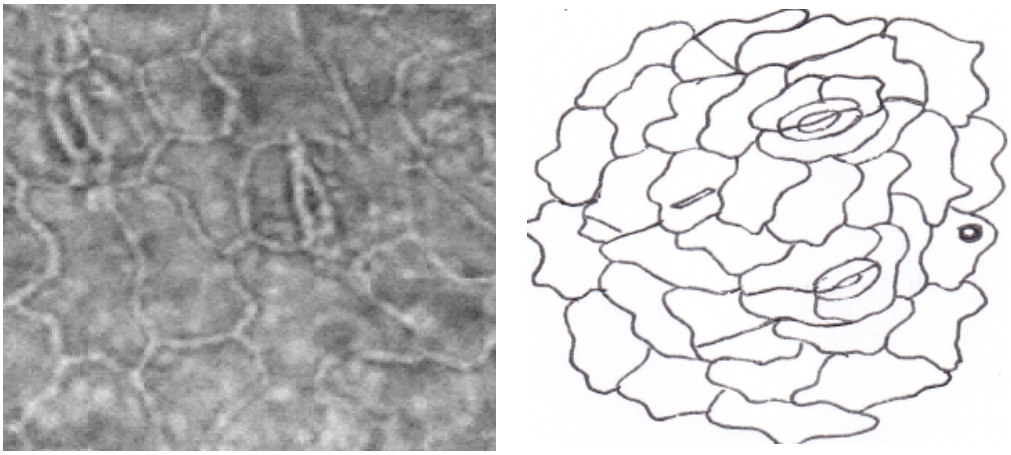

Fig. 17. Stomata are going to develop in *Trewia nudiflora*

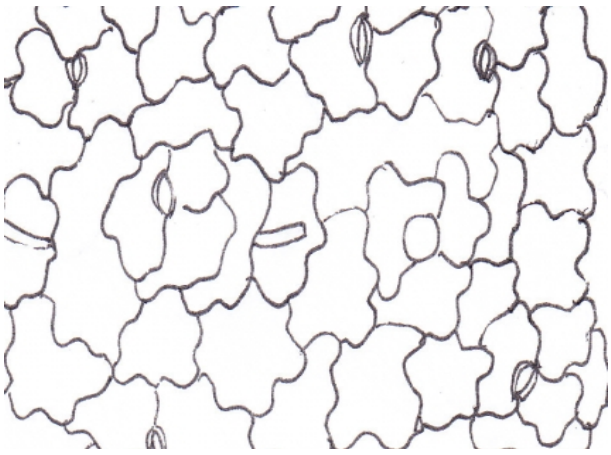

Fig. 18. Developed stomata and stomatal pattering in *Eryhrina subrosa*

4. DISCUSSION

A variation of mature stomata types and pattern were studied in tropical and subtropical arboreal species and their characteristics are shown in Table 1. Epidermal cells are observed to be straight in all the species. Five different shapes of the epidermal cells wall patterns are examined [16]. Revealed the number of micro morphological characters at taxonomic level in selected taxa of the genus *Ficus*L. (*Moraceae*). Irregular type of cells was found in *Pongamia glabra, Saraca asoca*, *Syzygium cumini*, *Sygzgium aromarticum*, *Trewia nudiflora*, *Thevetia peruviana* and *Albezia lebbeck*. Pentagonal epidermal cells are found in *Alstonia scholaris*, *Azadirachta indica* and *Lagerstroemia indica*. [25] investigated the pentagonal, rectangular and hexagonal types of cell and abundant stomatal types in *Solanu macrocarpon* and *Solanum nigrum.* For instance, many of the epidermal cells were observed to be rectangular in some dicot like *Murraya paniculata*, *Melia azedarachta*, *Kigelia pinnate*, *Eucalyptus camaldulensis* and *Acacia arabica*. Polygonal shape of epidermal cells were found in *Murraya koenigii*, *Magnolia grandiflora*, *Mangifera indica*, *Putranjiva roxburjh*, *Ficus racemosa*, *Ficus glomerata*, *Ficus infectoria*, *Erythrina subrosa* (Fig 18), *Dalbergia sisso*, *Callistemon lanceolatus* and *Sterculia alata* [26]. Studied cuticular characters in three species of *Dalbergia*. They distinguished the papillate and non papillatecuticular characters in two species. They just emphasized on the epidermal cells and ignored other anatomical characters. Whereas wavy shapes of epidermal cells were observed in many species and many were hexagonal such as *Averthoa caranmbola*, *Artocarpos integrifolia*, *Cassia fistula* and *Ficus religiosa*.

There was an apparent variation in stomatal index among the dicot species and their values did not appear to be influenced by the size or portion from which the peel was removed. This observation agrees with the finding of [9]. In which they added the variations in stomatal index are not influenced by the environment. This implies that their difference could be purely attributed distinction. The highest value of stomatal index was recorded 47.05 in *Syzygium cumini* L. The lowest value of stomata index was 1.92 in *Magnolia grandiflora* L.

In present study eight different types of stomata were recognized in tropical and subtropical arboreal species. Whereas [27] recognized seven (anisocytic, amphianisocytic, axillocytic, anomotetracytic, actinocytic, diacytic, staurocytic) types of stomata. They just emphasized on stomata anatomical marker but they ignored other anatomical character. Anomocytic stomata were present on both abaxial and adaxial surface in *Callistemon lanceolatus* and *Azadirachta indica*. This type of stomata is only present on abaxial surface of *Ficus infectoria* and *Ficus racemosa* [28]. Recognized six type of stomata (anomocytic, paracytic, diacytic, parallelocytic, cyclocytic and anisocytic). In which anomocytic stomata are most dominant found in 54 taxa of 69 dicot species. Amphianisocytic stomata were present on both abaxial and adaxial surface of *Pongamia glabra*. Brachyparacytic stomata were present on both abaxial and adaxial surface in *Averthoaparanmbola*, *Albezia lebbeck*, *Eucalyptus camaldulensis* and *Putranjiva roxburjhi*. On abaxial surface, brachyparacytic stomata are present in *Saraca asoca* and *Lagerstroemia indica*. Anomotetracytic stomata were observed on both abaxial and adaxial surface in *Murraya paniculata*, *Melia azadirachta*., *Magnolia grandiflora*, *Kigelia pinnate*, and *Syzygium cumini*. Because *Syzygium cumini* is medicinally important too. This species is exposed to clustering of stomata due to absoption of CO2. These stomata were present only on abaxial surface in *Sterculia alata*, *Mangifera indica*, and *Ficus glomerata*.

Table 1. Variation of stomatal index in the dicot species (30 species)

Species Name	ST	SI	SL	SW
Acacia arabica, Stewart	Anisocytic	20	58.1±18.7	18.0±7.0
Alstonia scholaris,R.Br	Anisocytic	3.12	66.4±31.7	12.6±8.2
Albezia lebbeck .L(bent).	Brachyparacytic	4.25	30.5±23.0	10.1±6.9
Artocarpos integrifolia spreng	Anisocytic	2.72	57.9±32.5	26.4±11.7
Averthoa caranmbola L	Brachyparacytic	8.53	35.5±20.5	23.3±6.9
Azadirachta indica L.	Anomocytic	5.26	45.3±21.1	18.4±10.4
Callistemon lanceolatus, DC	Anomocytic	9.83	39.7±18.0	10.8±6.7
Cassia fistula Linn.	Anisocytic	9.09	59.2±31.9	20.2±11.3
Dalbergia sisso, Roxb	Anisocytic	9.09	41.2±18.8	12.2±7.9
Erythrina subrosa ,L	Staurocytic	16.36	30.1±18.0	11.2±7.9
Eucalyptus camaldulensis Dehnh.	Brachyparacytic	8.69	67.9±35.5	18.4±13.4
Ficus racemosa Roxb	Anomocytic	3.2	59.1±44.1	14.6±11.2
Ficus religiosa, L	Staurocytic	45.45	42.3±25.1	15.0±9.1
Ficus infectoria Roxb. sana	Anomocytic	11.6	54.5±36.5	22. 5±15.3
Ficus glomerata, Roxb	Anomotetracytic	10.95	41.2±29.4	22.0±13.5
*Kigelia pinnate (*Jacq.*)*	Anomotetracytic	8.45	58.1±18.7	18.0 ±7.0
Lagerstroemia indica, L	Brachyparacytic	4.25	46.0±33.3	14.8±9.8
Magnolia grandiflora L	Anomotetracytic	1.92	31.0±20.0	10.0±6.0
Mangifera indica, L	Anomotetracytic	7.07	57.2±30.9	22.4±12.4
Melia azedarachta, L	Anomotetracytic	4.25	39.6±16.4	17.1±8.0
Murraya koenigi, L	Anisocytic	9.09	57.0±33.2	15.2±9.4
Murraya paniculata, L	Anomotetracytic	8.92	50.6±23.1	18.0±7.0
Pongamia glabra, Vent, Roxb	Amphianisocytic	5.17	50.0±26.9	15.3±9.3
Thevetia peruviana (pers).k schum	Anomotetracytic	9.09	51.5±26.2	25.5±14.6
Putranjiva roxburjhi wall.tent.FI	Brachyparacytic	5.40	31.4±17.1	10.8±9.1
Saraca asoca, L	Barachyparacytic	1.57	38.5±31.4	17.3±13.3
Sterculia alata Roxb	Anomotetracytic	21.05	30.7±19.4	7.3± 6.3
Syzygium cumini, L	Anomotetracytic	47.05	41.0±22.4	29.0±15.0
Sygzgium aromarticum L	Actinocytic	4.61	39.0±22.5	18.05±12.3
Trewia nudiflora L	Amphicyclocytic	5.86	85.9±52.1	44.5±22.5

Key: ST= Stomatal type, SI= Stomatal index (µm), SL= Stomatal length (µm) and SW= Stomatal width (µm)

On abaxial surface, actinocytic stomata were present in *Sygzgium aromaticum*. Staurocytic stomata are present on both abaxial and adaxial surface of *Ficus religiosa*.On abaxial surface this type of stomata was showing of stomatal patterning in *Erythrina subrosa*. *Anisocytic* stomata were present on both abaxial and adaxial surface in *Murraya koenigi*, *Dalbergia sisso*, *Cassia fistula* and *Acacia arabica*. On abaxial surface, this type of stomata was found in *Alstonia scholaris* and *Artocarpos integrifolia*. *Amphicyclocytic* stomata were present on both abaxial and adaxial surface of *Trewia integrifolia*. In this study Stomata play a vital role in the ability of land plants to balance water loss with photosynthetic performance. It has been known for decade that stomatal pattern alters in response to the environment.

4.1 Stomatal Patterning in Leaves

Dicotyledonous leaves exhibit fundamentally different modes of growth and development. While Monocot leaves have polarized growth from a single point source of cells at least near the leaf base, creating a leaf blade with the oldest cells at the tip. The epidermal consists of regular longitudinal files of cells, whose cells differentiate basipetally providing a belt of stages along the blade length. On the other hand, Dicot leaves grow from multiple point sources in a patch work like in quilt fashion, with clones of new cells forming throughout growth and development. At maturity, the epidermis consists of an irregular shaped cell

interspersed with stomata. Existence for this type of growth is based on marked mesophyll cells [29] and it seems likely that epidermal cells growth is also clonal in order to keep pace with division in the underlying cell layers. Pattern of new cell originated in dicot leaves from the basipetal of cells expansion [30]. This subsequent expansion complicates understanding stomatal patterning.

The mature leaf epidermis generally in dicot plants consists of three cells types: trichomes, stomatal guard cells and pavement cells. In dicot plant, trichomes are the first cells to differentiate and develop from leaf tip to base manner [6]. Stomata are developing in a basipetal manner and can form on both leaf surfaces or be restricted to one surface only (hypostomatal patterning). Their differentiation is the last aspect of leaf development [6,31]. In dicot, the stomata are usually scattered on leaf epidermis and separated from one another by a stomata-free region, which inhibit direct contact between the stomata [1,5,6]. Because the meristemoid cell may divide with two division. Asymmetrically divisions that produce a new large sister cell that has gone to cell fate. On the other hand these cell may divide symmetrically and produce a new two sister cell that turn to cell plastic to fate. Due to which these division causes the different types of stomatal patterning in the leaf epidermis that also dependent on species.

In most cases, stomatal density is the greatest on the abaxial surface, which may help to prevent water loss since the abaxial surface is less exposed to heating [32]. Whereas stomatal density, is lesser on the abaxial surface. It means to show that there is no cell division in species. In dicotyledonous plant stomatal patterning appears to be relatively random manner [33]. Such studies have observed the relationship between stomatal patterning and the arrangement of cells in the underlying layers.

Several mechanisms have been proposed as theories responsible for the origin and distribution of stomata cell lineage, inhibition and cell cycle. [34] established the cell lineage theory based on the monocotyledon epidermis .In this tissue; stomatal walls are staggered with respect to one another (Fig. 1). This theory contrast with the inhibition theory in the dicotyledons in which a series of divisions occur before the formation of stomata. A variable number of division in meristem cells of a cell field generates stomata in dicotyledonous. Cell linage and division are obvious components of every patterning mechanism. Bunning also advanced a theory of stomata patterning for dicotyledons which postulates that existing stomata prevent the formation of new ones by establishing a field of inhibition. The field has spatial limits so that when enough growth occurs and the effectiveness of the field is exceeded.

4.2 Stomatal Formation and Patterning

New stomata developed between mature complexes in dicotyledonous leaves and hypothesized that mature stomata released as in inhibitor to prevent new stomata from arising nearby [35]. Only when new growth exceeded the inhibitory influence would new stomata arise. Beyond Bunning's original observation, no measurements have established the size of the inhibitory field in any species and no inhibitor has been isolated. The scheme of stomatal typology [23,36] are based on the presence or absence of subsidiary cells relative to guard cells, and ancestral origin of cells within stomatal complexes. In dicotyledonous, the classification of the stomatal patterning based on shapes and arrangement of subsidiary cells.

Dicot stomata formation is scattered in time and space during the mosaic growth of the leaf. Studies of *Arabidopsis* leaf cells through time have been made it possible to distinguish between rules of stomatal development that are fixed and those that are flexible .All stomata form through at least one asymmetric and symmetric division. The first division takes place in a persumed stem cells that has become committed to the stomatal pathway, the meristomoid mother cell (MMC).This asymmetric division produces a small precursor, a meristomoid (CM), which is eventually converted into a guard mother cell (GMC). The symmetric division of the GMC produces the two guard cells that make up the stoma .Thus the terminal differentiation of a stoma occurs after a progressive through the three types of precursor cells (MMC to M to GMC).

The two types of development plasticity occur in the way that are related to the behaviour of the smaller and larger daughter cells produced by asymmetric division. Both types of plasticity contributed to epidermal formation and to stomata distribution. The first type of development plasticity concerns whether or not the smaller cell. The meristemoid cell divides asymmetrically, before assuming GMC fate .Meristomoid cells that are divided twice after their initial formed. That kind of division produces the different stomatal pattering in dicot.

4.3 Arrangement of Stomata

The orientations of stomatal pores is the most often parallel to long axis of the leaf .Although some exception exist [7,2]. Although there are a number of ways to acess stomatal distribution on leaves typical method include determination of stomatal frequency on an area basis and on a cell or an index basis on the adaxial sides of the leaf, the stomatal densities of adult leaves resemble. Those on the abaxial surface of the bilateral juvenile leaves. Isobilateral leaf anatomy is an adaptation too hot too dry condition. Differences in stomatal density are likely to be under the control of polarity derterming genes.

4.4 Patterning of the Stomata and Effect of the Environment

Plants are affected by the environment during all phase of growth and development. Especially stomatal number reportedly changes when plants are grown in different environment. Although the measurement are of the done on an area basis which ignores possible to effect resulting from changing cell size. It seems likely that the stomatal patterning mechanism may operate and respond to a range of condition. An inflexible rigid mechanism would offer little chance for evolutionary success. [37,38] have provided an evidence that stomata frequency decline in response to increasing CO2 and may have occurred over geological time. Stomatal frequency in present day can be estimated by growing them at different CO2 concentration [39].

Although some species undergo no change in patterning [40]. The absence of patterning change may indicate that some species are less sensitive to CO2 and or normally produce stomata in excess. There is an evidence of the latter in *Arabidopsis* where 30% of the stomata end bindley (with no stomatal chamber and would not function.

Since the stomatal aperture can be adjusted in vascular plants. So the advantage would result to a plant with a fewer stomatal growing in less CO_2. Due to which the less energy would be expended producing the specialized cells. The patterning mechanism would have to adjust stomatal distribution without impairing leaf function change occur in an internal leaf anatomy of each species to maintain an adequate gas exchange the pathway of perception

and response to elevated CO_2 is undoubtedly a complex and stomatal patterning plays a central role in the selected species. Another major factors known to affect the stomatal frequency are water and sunlight an extensive list of factors affecting stomatal propertied and their distribution on leaves has complied by [41].

5. CONCLUSION

Present study was conducted for taxonomic evaluation of the selected thirty arboreal species of dicot flora belonging to eight orders and fifteen families. Anatomical observation showed variation in size and shape of epidermal cells, cell arrangement variation in stomata types, size, and number and observed the patterning during the development of stomata. Leaf epidermal anatomy was found to be important tool for identification of dicot species. Within dicot flora, eight different shapes of stomata are observed (anomocytic, anomotetracytic, actinocytic, amphianisocytic, brachyparacytic, anisocytic, amphicyclocytic and staurocytic). Anomocytic stomata are most dominant important in arboreal species of dicot. Staurocytic stomata are found in *Ficus religiosa* L and *Erythrina subrosa* L. According to epidermal anatomical marker showed the different mode of stomatal patterning .It is observed that the environment also has significant effect on stomata development .In a number of species both light and CO2 concentrations have been shown to influence the stomata frequency at which stomata develop on leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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