Light and Scanning Electron Microscopic Investigation of Selected Taxa of Fabaceae and its Utilization for Taxonomic Classification

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Abstract- Light and scanning electron microscopy were conducted to study the leaf anatomical features of fourteen species of family Fabaceae. The plant species were *Acacia modesta*, *Albizia lebbeck*, *Bauhinia purpurea*, *Bauhinia variegata*, *Butea monosperma*, *Cassia fistula*, *Cassia javanica*, *Dalbergia sissoo*, *Erythrina herbacea*, *Pongamia pinnata*, *Prosopis juliflora*, *Robinia pseudoacacia*, *Sesbania sesban* and *Sophora secundiflora*. Scanning electron microscopy confirm that the stomata found in studied members are paracytic, tetracytic, anisocytic, and animocytic while the shape of epidermal cells are polygonal, elongated, tetragonal, wavy, and irregular type. Microscopic study shows that the maximum number of stomata were found on abaxail surface than adaxial surface. Paracytic type of stomata were found in majority of species while animocytic stomata found only in *Dalbergia sissoo*. Different types of stomata were found on both the surface of Fabaceae members but the stomata type is same on both surface of *Butea monosperma*, *Dalbergia sissoo*, *Erythrina herbacea*, *Prosopis juliflora*, and *Sesbania sesban*. *Sophora secundiflora* have highest number (39) of stomata on lower epidermis while *Bauhinia purpurea* L. and *Bauhinia variegata* L. have 5 stomata on lower epidermis. On the upper epidermis of *Sesbania sesban* have 5.33 number of stomata while 2 stomata were found on upper epidermis of *Bauhinia purpurea* L., *Butea monosperma* L., and *Dalbergia sissoo* Roxb. The *Pongamia pinnata* have highest (83%) stomatal index while *Erythrina herbacea* have lowest (10.77%) stomatal index on upper epidermis. In *Acacia modesta* have high (28.6%) stomatal index and *Prosopis juliflora* have low (10.32%) stomatal index on lower epidermal surface. The study of these anatomical features provide us a valuable taxonomic information for the identification of problematic taxa.

Index Terms- Fabaceae, Light Microscopy, SEM, Taxonomic significance, Stomatal index

I. Introduction

Fabaceae, a third largest family after Asteraceae and Orchidaceae with widely distributed species in all possible habitats including dry forest of Africa, America, and also a record number of species found in tropical rainforest [1]. The study of [2,3] authenticated that there are 730 genera and 19,400 species of Fabaceae. The species of this family are distributed in subtropical areas, grassland, wood grassland, temperate, and sub-temperate regions of Pakistan. Maximum species are grown in dry grassland habitats [4]. Family Fabaceae were classified into three subfamilies. This traditional classification of family Fabaceae includes the Caesalpinioideae, Mimosoideae, and Faboideae [5-7]. The key feature of the members of this family is to form Symbiotic and ectomycorrhizal association with bacteria and fungi respectively. Therefore, the Fabaceae species considers as high economic value and ecological curiosity [8]. Economically, most species are used as food, fodder, ornamental, and raw material for industry [5]. The members which we utilize as a food and for agriculture practice are *Medicago sativa* L., *Glycine max* L. Merr., *Phaseolus* species, *Pisum sativum* L., and *Cicer arietinum* L. The *Arachis hypogaea* L. seeds contain essential oil which are utilized for the purpose of cooking [9]. The medicinal plants include *Mucuna pruriens*, *Crotalaria albida*, and *Glycyrrhiza glabra* are used to treat various diseases. The Species of *Butea frondosa*, *Lathyrus odoratus* and *Lupinus hirsutus* are cultivated as ornamental plants [2]. The anatomical features of leaf are implemented in plant systematics for over the last 100 years. Leaf anatomical studies is a tool which put the species in correct taxonomic position and to identify various species [10-12] The traditional classification of plants based on morphological features of fruits and flowers, while now taxonomist identify plants with the help of leaf epidermal features[13-17]. Epidermal characteristics of leaf have great contribution to find out solution of taxonomic problems [18,19]. Epidermal morphological features were followed many researchers to study the accurate taxonomic rank of any botanical taxa. Many authors in their taxonomical studies of botanical reviews. Some of the researchers [20-24] considers leaf epidermal characters to solve effectively the taxonomic problems [25]. The leaf epidermal studies are significant because the leaves are present over for longer time such as fossil record of dead and fragment remains of drug-based species which provide us valuable information about the production of taxonomic principles [26]. Leaf epidermal characters play an important role

for creation of taxonomic monograph. The identification of different groups of plants were authenticated with the help of leaf epidermal features [27,28]. The study of [29] stated that the epidermal cells shape, size, and pattern are of significant role for sorting phylogenetic relationship among plants. Taxonomic studies are incomplete without the consideration of leaf epidermal features. The consideration of micro-morphological features in taxonomic studies are important to delimit many plant species [30-33].

II. MATERIALS AND METHODS

2.1 Collection, identification, and herbarium deposition

Different areas of the target research sites were visited and collected 14 species of family Fabaceae. These collected species with the help of relevant literature [34-39] and flora of Pakistan [40-43] were identified, pressed and then mounted on herbarium sheets. The voucher number were assign for each species and deposited at Centre of Plant Biodiversity, University of Peshawar.

2.2. Study of foliar epidermis using LM

Foliar epidermal studies were conducted under the light microscope. The technique of Cotton [44]; Clark, [45], Gul et al. [46] were applied to prepare the leaf samples from fresh and dried leaves for LM studies. Fresh leaves were treated with water for about 2 hours in order to keep them moistened. The peeling method were utilized for removing the epidermis strips. In solution of CH_3COOH, the leaves were heated for 15 minutes to remove the epidermis from both the surface of leaf. The leaves having thick cuticle were dipped in Farmer's fluids with 1:1:3 percentages and then they were stored in 70% alcohol. First, the glycerin jelly was applied to the epidermal layers and then epidermal layer were stained with the help of Delafiel's Hematoxylin. In order to obtain accurate data, three slides for each species were prepared and observed its anatomical features under light microscope. The images of the samples were taken with the help of polarized camera in the Lab of Botany Department, University of Peshawar. Various micro-morphological features of both the epidermal layer of leaves were observed using 10x and 40x lens.

2.2. Investigation of foliar epidermis using SEM

The qualitative features including type of stomata and epidermal cell shape were studied under SEM at Central Resource Library, University of Peshawar, Pakistan. To remove the epicuticular wax from both the surface of leaves, the dried and pressed leaves were soaked in xylene (C8H10) for 12 to 24 hours. Then the leaves were placed on metallic stubs with the help of scotch tape and wrapped it by using the sputter-coated with gold palladium inside sputtering chamber.

2.4. Statistical Analysis

Quantitative features including number of stomata and number of epidermal cell were analyzed through statistical tool to get Mean and Standard deviation. The following equation described by Salisbury (1928, 1932) was followed to calculate the stomatal index.

$$S:I = \frac{S}{E+S} \times 100$$

S:I represent the stomatal index, S= Stomata number per unit area, and E= Epidermal cell number per unit area.

III. RESULTS

Foliar epidermal anatomy

The leaf anatomy were studied with the help of light and scanning electron microscopy to find out the significant variation in quantitative and qualitative characters of 14 species of family Fabaceae. The results of the investigated taxa were summarized in Table 1, 2 and 3.

Epidermal cell morphology

The morphological features of epidermal cells are presented in Figure 1. Which shows that the difference in epidermal cell shape are significantly important and act as taxonomic tool for delimitation of taxa. Epidermal cells are found to be irregular in the *Cassia javanica* (14) and *Sesbania sesban* (25,26). Other species have polygonal, elongated, tetragonal and wavy type of cells. The polygonal epidermal cells are found in *C. fistula* (11,12) and *C. javanica* (13,14). While tetragonal cells are present in *A. modesta* (1) and elongated cells are found in *B. purpurea* (5) and *D. sissoo* (15).

Micromorphology of Stomata

This result shows that the paracytic stomata found in many member of family Fabaceae while anisocytic type of stomata are less frequently observed in investigated taxa. We found in this result that the stomata are widely distributed on the abaxial surface in investigated species than the adaxial surface of epidermis. The stomata shape varies from Paracytic, Tetracytic, Anomocytic to Anisocytic. Paracytic type of stomata were found in most of the investigated species. Table 1 shows that the *Dalbergia sissoo* Roxb was only the investigated taxa which have two different stomata on both the epidermal layer while similar type of stomata are found on both the surface of *Prosopis juliflora* (Swartz) DC, *Erythrina herbacea* L. and *Butea monosperma* L. *Sesbania sesban* (L.) Merr have only anisocytic stoma on both surface of epidermis. The stomata were absent on the adaxial surface of *Acacia modesta* Wall., *Albizia lebbeck* (L.) Benth, *Cassia fistula* L., *Cassia javanica* L., *Pongamia pinnata* (L.) Pierre and *Sophora secundiflora* (Ortegal) DC. While the rest of species have stomata on the abaxial surface. On both the epidermal layer of *Erythrina herbacea* L., *Butea monosperma* L. and *Prosopis juliflora* (Swartz) DC. have paracytic type of stomata except *Pongamia pinnata* (L.) Pierre., *Sophora secundiflora* (Ortegal) DC and *Acacia modesta* Wall. Which have only paracytic type of stomata. Paracytic and Tetracytic stomata were observed in *Albizia lebbeck* L., *Cassia fistula* L. and *Cassia javanica* L. The Anisocytic and paracytic type of stomata were found in *Bauhinia purpurea* L. and *Robinia pseudoacacia* Poir.

IV. DISCUSSION

In this study we report the foliar anatomical features of family Fabaceae. The key features of Fabaceae were focused in this study are stomatal types, epidermal cell shape, and number of epidermis cell. The finding of this study is to classify the plant species on the basis of foliar epidermal characters and to provide us valuable taxonomic information to expose the key roles of micro-morphological study in the classification of plant species. The leaf condition of family Fabaceae members have both hypo-amphistomatic [29,46] and epidermal cells are found to be irregular, rectangular, and isodiametric [46]. Scanning electron

microscopy technique were utilized with the aim to further emphasize and observed the distinguish features of foliar epidermis of family Fabaceae. We found that the lower surface of the leaves of family Fabaceae are amphistomatic type while the upper surface is hypostomatic type like the results of [47,48]. Based on this study our finding confirms that the anatomical characters are useful for the differentiation of many problematic genera and species of various plants and also delimit the plant taxa [49]. The study of [50] stated that the size and shape of epidermal cell on abaxial and adaxial surface of leaf have important role in the classification of plants. Similar type of stomata is found on both surface of leaf in *Butea monosperma* L., *Dalbergia sissoo* Roxb. *Erythrina herbacea* L., *Prosopis juliflora*, and *Sesbania sesban* (L.) Merr. While stomata were found different on both the surface of *Robinia pseudoacacia* Poir, *Bauhinia purpurea* L., and *Bauhinia variegata* L. Most of the species possess paracytic type of stomata while animosytic stomata are rarely present in all studied members[29]. The anisocytic stomata found on both the surface of *Sesbania sesban* (L.) leaves confirmed the similar finding of [51]. In study of [52] supported our work that the stomata are widely distributed on the abaxial surface than adaxial surface of leaves. The result revealed that the variation found in epidermal layers of all 14 species are significant for their species delimitation and indicate the genetic diversity among the members of Fabaceae family. Generally, the high stomata indices were observed on abaxial surface than the adaxial surface is a common feature in the investigated species of Fabaceae family which confirms taxonomic importance and over-emphasized the role of stomatal index in plant systematics. The separation of plant species on the basis of stomatal index cannot be over-considered by many researchers [53]. High number of epidermal cells found on abaxial surface of leaves in studied members is an important taxonomic and evolutionary features because the epidermal cells number less than 250 in plant species on abaxial surface were considered together in same group. The epidermal cell shapes are polygonal, irregular and wavy type. The similar result were also reported by [51] which support our present work. We specially emphasize in this study the role of stomatal index in distribution of taxa which were not over-emphasized as a tool in taxonomic study so far. Further studied were appreciated to explain the anatomical features of family Fabaceae with special reference to the stomatal index.

V. CONCLUSION

In this research project we studied the anatomical features of 14 species of family Fabaceae. Variation observed in morphological characters of epidermal surface confirms the role of anatomical study are great importance in classification of plants. We concluded from this study that the stomatal type and epidermal cell shape provide us a valuable taxonomic key which are helpful in the delimitation of taxa. Based on this key, taxonomist solve the problems and confusions occur in the identification and classification of problematic taxa. The present work concludes that the use of SEM helps a lot to distinguish the anatomical features of the species of same genus which is not possible under LM. Furthermore, stomatal index and epidermal cell shape broad our knowledge in the correct placement of taxa.

ACKNOWLEDGEMENT

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Table 1. Light and Scanning Electron Microscopic Studies of Stomata on Upper and Lower Epidermis of selected taxa of Fabaceae.

S. No.	Plant species	Stomata		Stomatal types		Stomatal index	
		Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis
1.	*Acacia modesta* Wall.	A	P	-	Paracytic	26	28.6
2.	*Albizia lebbeck* (L.) Benth.	A	P	-	Paracytic+ Tetracytic	16	25.92
3.	*Bauhinia purpurea* L.	P	P	Paracytic	Anisocytic +Paracytic	13.29	25
4.	*Bauhinia variegata* L.	P	P	Anisocytic	Tetracytic	12.46	11.90
5	*Butea monosperma* L.	P	P	Paracytic	Paracytic	14.25	28
6.	*Cassia fistula* L.	A	P	-	Paracytic+ Tetracytic	23	17.49
7.	*Cassia javanica* L.	A	P	-	Paracytic+ Tetracytic	32	18.75
8.	*Dalbergia sissoo* Roxb.	P	P	Anomocytic+Paracytic	Anomocytic+Paracytic	12.4	16.19
9.	*Erythrina herbacea* L.	P	P	Paracytic	Paracytic	10.77	20
10.	*Pongamia pinnata* (L.) Pierre	A	P	-	Paracytic	83	21.04
11.	*Prosopis juliflora* (Swartz) DC.	P	P	Paracytic	Paracytic	25.91	10.32
12.	*Robinia pseudoacacia* Poir.	P	P	Anisocytic	Anisocytic +Paracytic	11	25.92
13.	*Sesbania sesban* (L.) Merr.	P	P	Anisoytic	Anisoytic	21.74	23
14.	*Sophora secundiflora* (Ortegal) DC.	A	P	-	Paracytic	15	17.72

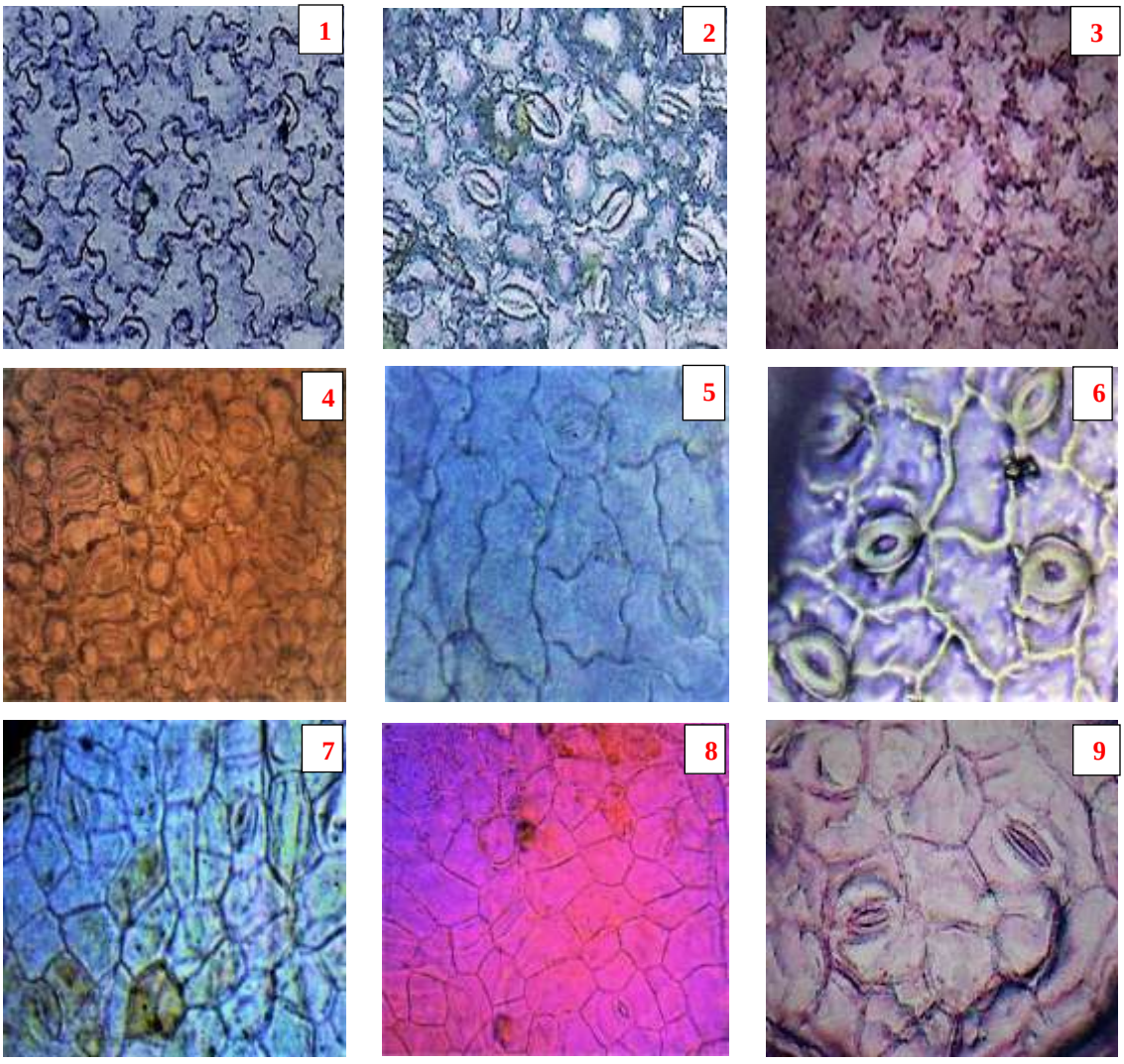
Table 2. Mean and Standard Deviation of the Upper Epidermis and Stomata of the 14 members of Fabaceae.

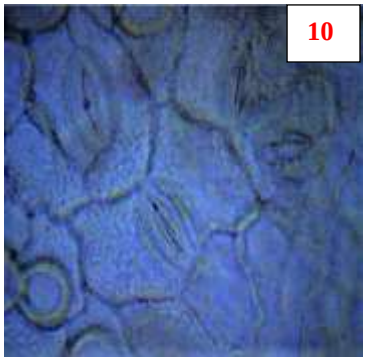
S. No.	Plant Species		Mean	Standard deviation
1.	*Acacia modesta* Wall.	Stomata	-	-
		Epidermis	27	2
2.	*Albizia lebbeck* (L.) Benth.	Stomata	-	-
		Epidermis	17	2
3.	*Bauhinia purpurea* L.	Stomata	2	1
		Epidermis	12.66	5.5
4.	*Bauhinia variegata* L.	Stomata	2.66	0.57
		Epidermis	20.66	4.04
5	*Butea monosperma* L.	Stomata	2	1
		Epidermis	12.33	1.52
6.	*Cassia fistula* L.	Stomata	-	-
		Epidermis	24.33	4
7.	*Cassia javanica* L.	Stomata	-	-
		Epidermis	33	4
8.	*Dalbergia sissoo* Roxb.	Stomata	2	1
		Epidermis	14	5.29
9.	*Erythrina herbacea* L.	Stomata	2	1
		Epidermis	16.33	5.50
10.	*Pongamia pinnata* (L.) Pierre	Stomata	-	-
		Epidermis	85	5
11.	*Prosopis juliflora* (Swartz) DC.	Stomata	14.33	4.72
		Epidermis	40	5
12.	*Robinia pseudoacacia* Poir.	Stomata	2	1
		Epidermis	16.33	1.52
13.	*Sesbania sesban* (L.) Merr.	Stomata	5.33	1.52
		Epidermis	18	6.24
14.	*Sophora secundiflora* (Ortegal) DC.	Stomata	-	-
		Epidermis	153	4

Table 3. Mean and Standard Deviation of the Lower Epidermis and Stomata of the 14 members of Fabaceae.

S. No.	Species		Mean	Standard deviation
1.	*Acacia modesta* Wall.	Stomata	9.66	2.08
		Epidermis	25	4.35
2.	*Albizia lebbeck* (L.) Benth.	Stomata	7	1
		Epidermis	19.66	4.04
3.	*Bauhinia purpurea* L.	Stomata	5	1
		Epidermis	15.33	3.21
4.	*Bauhinia variegata* L.	Stomata	5	1
		Epidermis	37.33	3.36
5	*Butea monosperma* L.	Stomata	7	1
		Epidermis	18.33	2.88
6.	*Cassia fistula* L.	Stomata	7	1
		Epidermis	33.3	2.08
7.	*Cassia javanica* L.	Stomata	6	1
		Epidermis	25.66	5.12
8.	*Dalbergia sissoo* Roxb.	Stomata	6.33	1.52

		Epidermis	31.33	4.15
9.	*Erythrina herbacea* L.	Stomata	6	1
		Epidermis	23.66	3.78
10.	*Pongamia pinnata* (L.) Pierre	Stomata	12	5
		Epidermis	45	5
11.	*Prosopis juliflora* (Swartz) DC.	Stomata	19.66	1.52
		Epidermis	165	5
12.	*Robinia pseudoacacia* Poir.	Stomata	6.66	1.52
		Epidermis	20.33	6.65
13.	*Sesbania sesban* (L.) Merr.	Stomata	6	1.22
		Epidermis	20	1
14.	*Sophora secundiflora* (Ortegal) DC.	Stomata	39	3.60
		Epidermis	74.33	4.04

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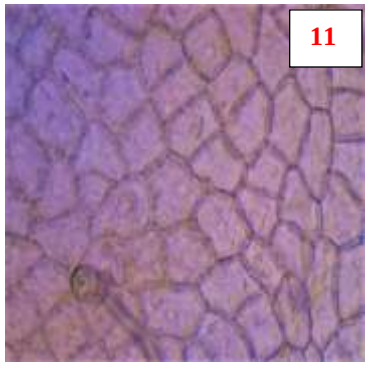
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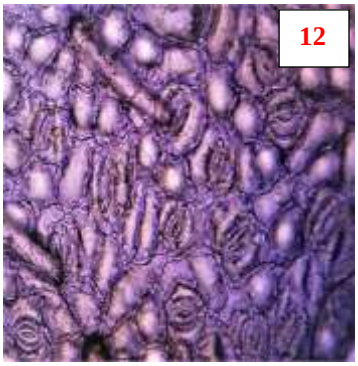
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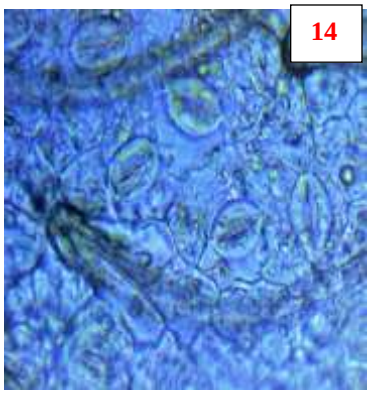
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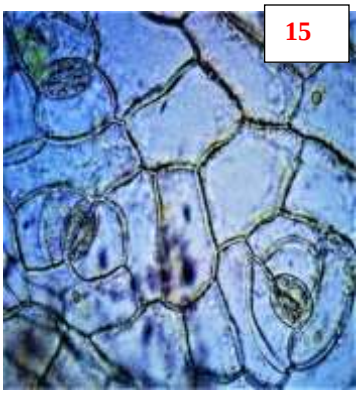
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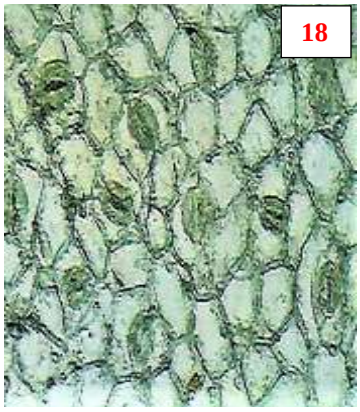
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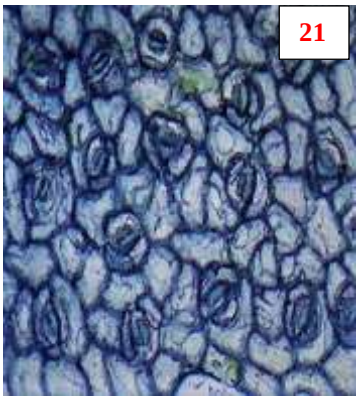
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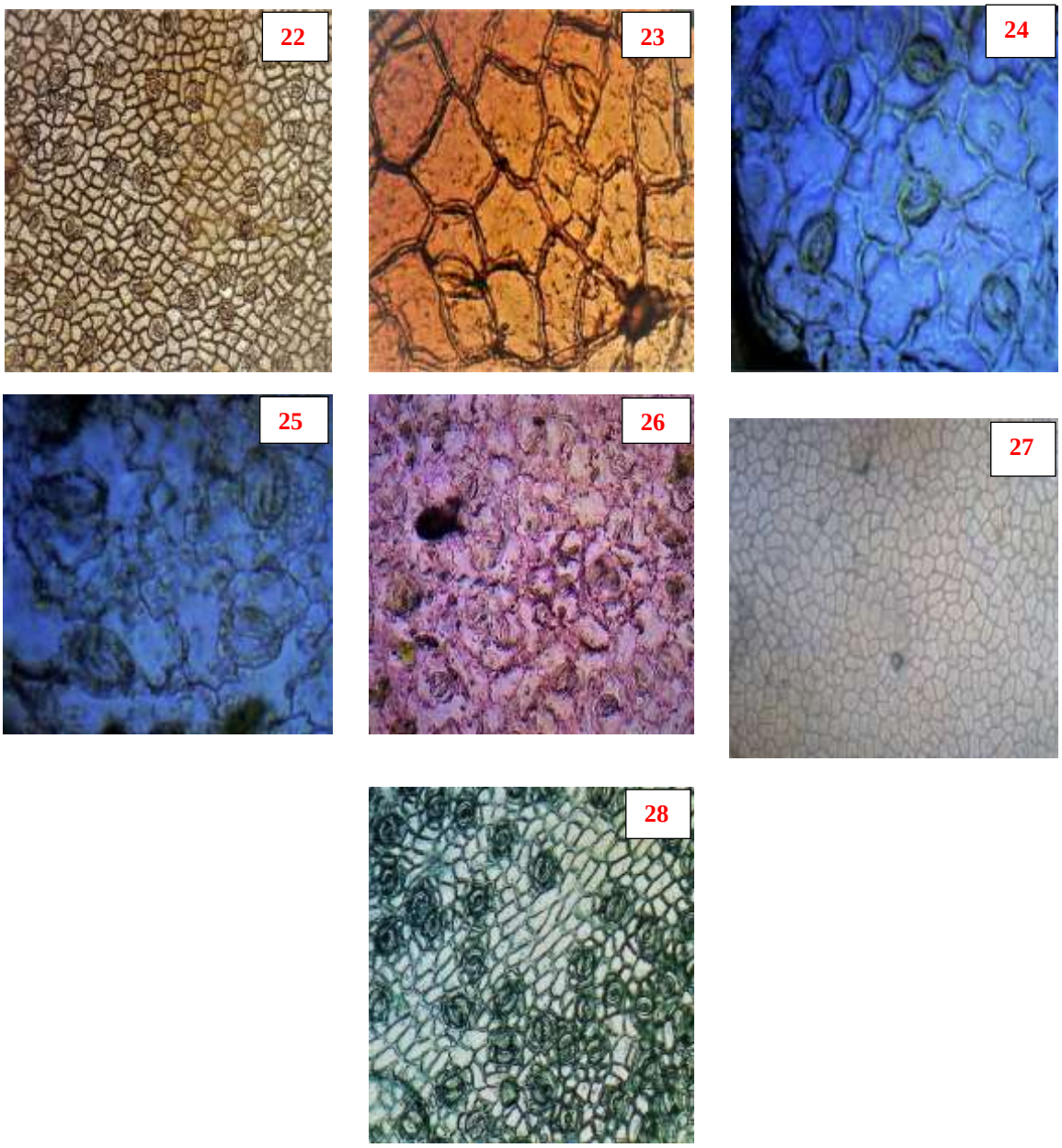

Figure 1. Light microscopy showing stomata structure of the species of Family Fabaceae; *Acacia modesta* (1,2). *Albizia lebbeck* (3,4). *Bauhinia purpurea* (5,6). *Bauhinia variegata* (7,8). *Butea monosperma* (9,10). *Cassia fistula* (11,12). *Cassia javanica* (13,14). *Dalbergia sissoo* (15,16). *Erythrina herbacea* (17,18). *Pongamia pinnata* (19,20). *Prosopis juliflora* (21,22). *Robinia pseudoacacia* (23,24). *Sesbania sesban* (25,26). *Sophora secundiflora* (27,28).

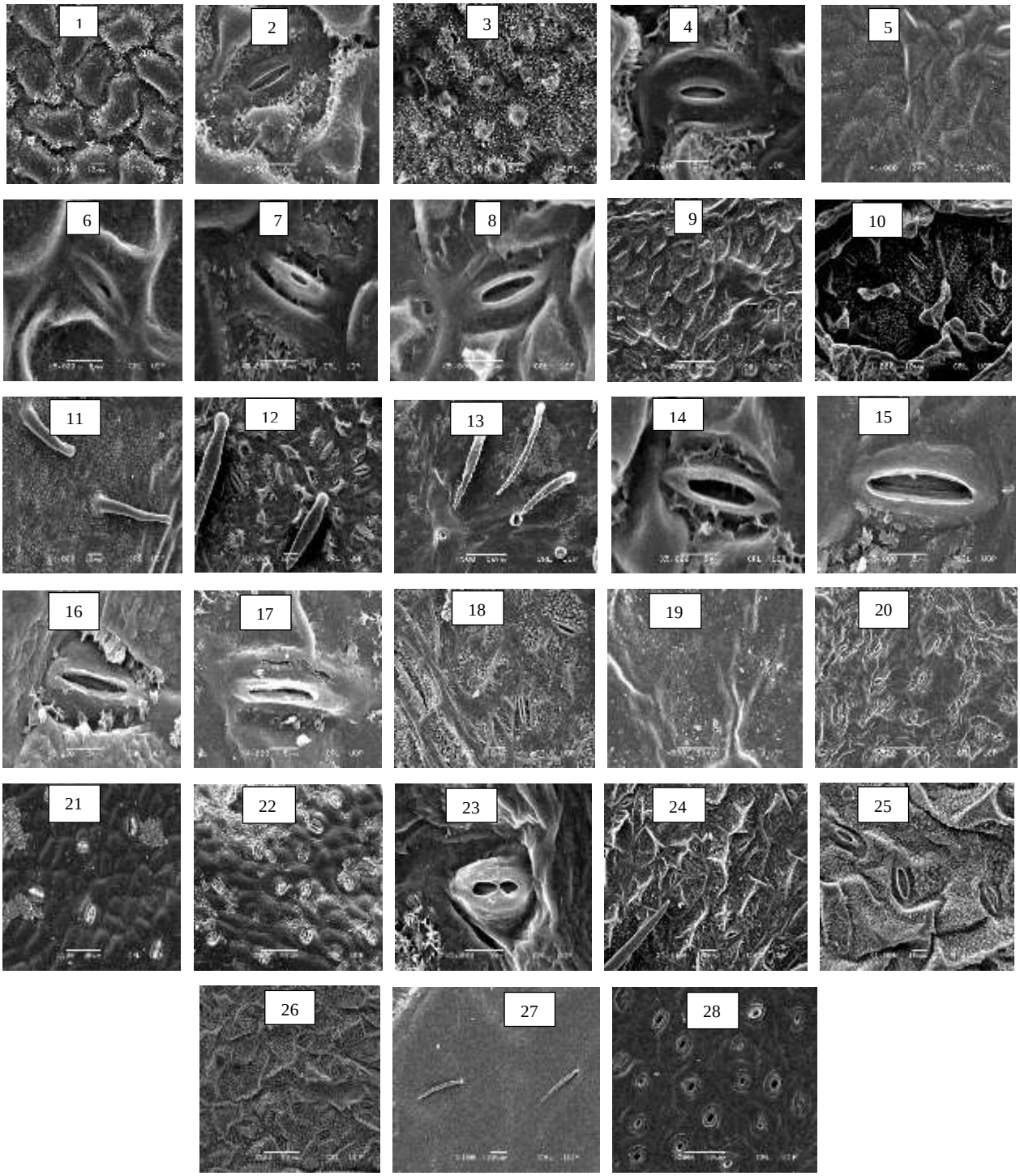

Figure 2. (1) Stomata of *Acacia modesta* × 1000 UE (2) Stomata of *Acacia modesta* × 2,500 LE (3) Stomata of *Albizia lebbeck* × 1000 UE (4) Stomata of *Albizia lebbeck* × 4,000 LE (5) Stomata of *Bauhinia purpurea* × 1,000 UE (6) Stomata of *Bauhinia purpurea* × 5,000 LE (7) Stomata of *Bauhinia variegata* × 5,000 UE (8) Stomata of *Bauhinia variegata* × 5,000 LE (9) Stomata of *Butea monosperma* × 500 UE (10) Stomata of *Butea monosperma* × 1,000 LE (11) Stomata of *Cassia fistula* × 1,000 UE (12) Stomata of *Cassia fistula* × 1,000 LE (13) Stomata of *Cassia javanica* × 500 UE (14) Stomata of *Cassia javanica* × 5,000 LE (15) Stomata of. *Dalbergia sissoo* × 5,000 UE (16) Stomata of. *Dalbergia sissoo* × 5,000 LE (17) Stomata of *Erythrina herbacea* × 4,000 UE (18) Stomata of *Erythrina herbacea* × 1,000 LE (19) Stomata of *Pongamia pinnata* × 500 UE (20) Stomata of *Pongamia pinnata* × 500 LE (21) Stomata of *Prosopis juliflora* × 500 UE (22) Stomata of *Prosopis juliflora* × 500 LE (23) Stomata of *Robinia pseudoacacia* × 5,000 UE (24) Stomata of *Robinia pseudoacacia* × 1,000 LE (25) Stomata of *Sesbania sesban* × 1,000 UE (26) Stomata of *Sesbania sesban* × 500 LE (27) Stomata of *Sophora secundiflora* × 100 UE (28) Stomata of *Sophora secundiflora* × 500 LE

Key Based on Stomatal types, Absence or Presence and Stomatal index

1. + Stomatal types, absent or present ……..… 2
   - Stomatal index ……..7
2. + Stomata absent on upper surface ..... *Acacia modesta*
   - Stomata present on upper surface ..... (3)
3. + Stomata anomocytic and paracytic on upper surface…… *Dalbergia sissoo*
   - Stomata anisocytic on upper + lower surface ……..... (4)
4. + Stomata anisocytic on upper surface ….. *Sesbania sesban*
   - Stomata paracytic on upper+ lower surface …….... (5)
5. + Stomata paracytic on lower surface…….*Butea monosperma*
   - Stomata tetracytic on lower surface ….... (6)
6. + Stomata tetracytic on lower surface ……. *Bauhinia variegata*
   - Stomatal index above 32 on upper epidermis …….. (7)
7. + Stomatal index 83 on upper surface …. *Pongamia pinnata*
   - Stomatal index above 25 on upper surface …….. (8)
8. + Stomatal index 32 on upper surface …….. *Cassia javanica*
   - Stomatal index less than 32 on adaxial surface ….. (9)
9. + Stomatal index 25.91 on upper epidermis…….... *Prosopis juliflora*
   - Stomatal index above 15 on upper surface …… (10)
10. + Stomatal index 23 on adaxial surface ……....*Cassia fistula*
    - Stomatal index less than 13.29 on upper side ……..….(11)
11. + Stomatal index 12.46 on adaxial side…… *Bauhinia purpurea*
    - Stomatal index above 20 on abaxial side ……..… (12)
12. + Stomatal index 25.92 on abaxial surface ……...*Robinia pseudoacacia*
    - Stomatal index less than 25.92 on lower side ……..… (13)
13. + Stomatal index 20 on lower surface ……..... *Erythrina herbacea*
    - Stomatal index above 20 on abaxial surface …… (14)
14. + Stomatal index 21.04 on abaxial side ……..... *Pongamia pinnata*

Stomatal index 17.72 on lower side ……..... *Sophora secundiflora*

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