Research Paper

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Stomatal Variations Among Certain Wild Angiospermic Plants of Mysore, India

Biotechnology

KEYWORDS : Stomata types, index, aperture, frequency, Venn diagram

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ABSTRACT

Stomatal type, stomatal index, stomatal frequency and area of stomatal aperture of 11 wild angiospermic plants in a unique environmental region of Mysore district were studied. Out of the 11 wild species, 6 plants showed anomocytic type of stomata. 3 of them had paracytic type of stomata and 2 of them showed anisocytic stomata. Pongamia pinnata L, Ficus racemosa L and Tinospora cordifolia (Thunb.) Miers were hypostomatic (stomata only on the lower epidermis). The remaining 8 species were amphistomatic (stomata on both the epidermal surfaces). The data was subjected to the online Venn Diagram Plotter to derive sets of similar data. Large variations in stomatal type, stomatal index, stomatal frequency and area of stomatal aperture were observed, although diverse plants survived in a unique environment.

Introduction

Stomata are structures present in the epidermis of the leaves which primarily help in regulating the process of transpiration and gaseous exchange. The term 'stoma' refers to the stomatal opening plus the guard cell. These guard cells have a dense granular cytoplasm and some chloroplasts. The cell walls of the guard cells are unevenly thick and this plays an important role in the opening and closing of the stomata.

Leaves of angiospermic plants, exhibit two different modes of stomatal growth and development. In the dicotyledonous leaves, the stomata remains scattered, while in the monocotyledonous leaves, they occur in parallel rows. In general, stomata are more abundant in the lower epidermis of the dorsi-ventral leaf; stomata are more or less evenly distributed on all sides. (Dalal, 2012)

Fowden et al. (1993) reported that stomata perform the function of gaseous exchange which also includes the importance of photosynthesis and respiration.

Transpiration occurs mainly through the stomata in the leaves. Besides the loss of water vapour in transpiration, exchange of oxygen and carbon dioxide in the leaf also occurs through stomata (gaseous exchange). Hetherington et al. (2003) suggest that the gaseous exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis. Environmental signals such as light intensity, the concentration of atmospheric carbon dioxide and endogenous plant hormones control the stomatal aperture and development.

The stomatal types are distinctive of certain families such as Ranunculaceae (anomocytic), Brassicaceae (anisocytic), Caryophyllaceae (diacytic) and Rubiceae (paracytic). Anomocytic has ordinary epidermal cells surrounding the stomata. In others, the epidermal cells surrounding the stomata are differentiated as subsidiary cells. There maybe two subsidiary cells at right angles to the guard cells (diacytic), two or more cells parallel to guard cells (paracytic) or three subsidiary cells of unequal size (anisocytic). The stomatal complex of Poaceae is distinctive in having two dumb bell shaped guard cells with two small subsidiary cells parallel to the guard cells. Anjum Perveen et al. (2011) states that, the stomata can occur on both surfaces of the leaf (amphistomatic leaf) or on only either the upper (epistomatic leaf) or more commonly on the lower side (hypostomatic leaf).

Croxdale (2000) reports that since stomata are structures critical to the survival of terrestrial plants, they need to be viewed in relation to their function and their interface with other structural components. Two dimensional structures of stomata are well suited for investigation because the pattern simplifies analysis, and because stomata occur on organ surfaces, which make them readily accessible.

Materials and Methods

Selection of specimens

Eleven wild angiospermic plants were selected for this experiment from the Yelwal region of Mysore.

Isolation of epidermal imprints

For this purpose, the replica technique was used. Here, a film of clear nail polish was directly applied to the upper and lower surface of leaf. After allowing it to dry, the clear film was easily peeled off using clean forceps and micro needle. This synthetic impression produces an excellent replica of the epidermis and stomata. (Chen, 2001)

Microscopic study

A small portion of the synthetic stomatal imprint was mounted in a drop of water for microscopic study to determine the stomatal types, Stomatal index, frequency and area.

Stomatal type

Stomatal types were determined based on the classification of stomata on the grounds of nature and number of subsidiary cells. (Metcalfe & Chalk, 1950)

Stomatal Index

Stomatal Index (SI) was determined using the formula, given by Salisbury (1927):

$$SI = \frac{S}{E + S} X\ 100$$

Where,

S: Number of stomata per unit area.
E: Number of epidermal cells per unit area.

Stomatal frequency

The stomatal frequency was determined as percentage occurrence of each stomatal type in $1cm^2$ of the microscopic field at 40x magnification under Labomed microscope.

Area of stomatal aperture

The length and breadth of the stomatal aperture was measured using Axiovision software (in µm) and the area of the stomatal aperture was calculated using the formula:

$$A = \frac{\pi}{2} \times l \times bA = \frac{\pi}{2} \times l \times b\ \mu^2$$ (since it is a semicircle)

Statistical analysis
The Venn diagram plot was used to explain the variances in the stomatal types.

Results and Discussion
Details of the observations are presented in Table 1. Results of the Venn diagrams are presented in Figs 1.1 to 1.4. The different types of stomata are shown in Plate 1.

Out of 11 wild angiospermic plants, Pongamia pinnata.L, Ficus racemosa.L and Tinospora cordifolia (Thunb.) Miers exhibited hypostomatous condition i.e., stomata only on the lower epidermis. While the rest were amphistomatic i.e., stomata found on both the epidermal surfaces of the leaf.

Results based on the parameters studied are as follows:
Stomatal type

Anomocytic – Epidermal cells surrounding the stomata are not differentiated into subsidiary cells. The species included in this type are: Conyza stricta Less, Achyranthes aspera L, Pongamia pinnata.L, Ficus racemosa.L, Psidium guajava.L and Tinospora cordifolia (Thunb.) Miers.

Paracytic – These have two or more cells that are parallel longitudinally to that of the guard cell and are differentiated as subsidiary cells. The species included in this type are: Sida acuta Burm. f, Alternanthera pungens Kunth and Ipomoea sp.

Anisocytic – There exists three subsidiary cells of unequal size exist around the stomata. The species included in this type are: Plumbago zeylanica.L and Amaranthus viridis,L

Stomatal Index
It is reported that the maximum and minimum values of stomatal index on the upper surface were 28.26 (Amaranthus viridis,L) and 6.66 (Plumbago zeylanica.L) respectively. On the lower surface, the maximum and minimum values of stomatal index were 26.92 (Plumbago zeylanica.L) and 6.59 (Achyranthes aspera L) respectively.

Stomatal Frequency
The highest and lowest values of stomatal frequency on the upper surface were 2588 (Sida acuta Burm. f and Amaranthus viridis,L) and 398 (Plumbago zeylanica.L) respectively. On the lower surface, highest and lowest values were 5971 (Psidium guajava.L) and 995 (Tinospora cordifolia (Thunb.) Miers) respectively.

Length and Breadth of stomatal aperture
On the upper surface, the length of the stomatal aperture varied from 0.203 µm (Psidium guajava.L) to 0.428 µm (Ipomoea sp.). On the lower surface, length varied from 0.184 µm (Sida acuta Burm. f) to 0.561 µm (Tinospora cordifolia (Thunb.) Miers). Breadth of the stomatal aperture, on the upper surface extended from 0.042 µm (Conyza stricta Less) to 0.145 µm (Psidium guajava.L). On the lower surface, breadth varied from 0.027 µm (Alternanthera pungens Kunth) to 0.253 µm (Tinospora cordifolia (Thunb.) Miers).

Area of stomatal aperture
With respect to area of stomatal aperture, the highest and lowest values on the upper surface were 0.097 μm^2 (Ipomoea sp.) and 0.021 μm^2 (Sida acuta Burm. f) respectively. And on the lower surface, the highest and lowest values were 0.227 μm^2 (Tinospora cordifolia cordifolia (Thunb.) Miers)) and 0.012 μm^2 (Alternanthera pungens Kunth) respectively.

Table 1 - Showing stomatal variations among certain wild angiospermic plants of Mysore.

No.	Species	Stomatal type	Stomatal Index		Stomatal Frequency		Average length of stomatal aperture (µm)		Average breadth of stomatal aperture (µm)		Average area of stomatal aperture (μm^2)	
	Surface		U	L	U	L	U	L	U	L	U	L
1	Plumbago zeylanica L	Anisocytic	6.66	26.92	398	2787	0.366	0.371	0.134	0.182	0.082	0.108
2	Sida acuta Burm.f	Paracytic	13.54	15.91	2588	4578	0.269	0.184	0.050	0.053	0.021	0.015
3	Alternanthera pungens Kunth	Paracytic	10.81	12.5	1592	1194	0.268	0.272	0.094	0.027	0.041	0.012
4	Conyza stricta Less	Anomocytic	14.28	17.91	1592	2389	0.399	0.348	0.042	0.129	0.045	0.071
5	Achyranthes aspera L	Anomocytic	18.51	6.59	1990	1194	0.292	0.298	0.068	0.074	0.031	0.035
6	Pongamia pinnata.L	Anomocytic	00	17.5	00	1791	00	0.247	00	0.102	00	0.040
7	Amaranthus viridis.L	Anisocytic	28.26	21.57	2588	1990	0.299	0.234	0.048	0.078	0.023	0.029
8	Ficus racemosa.L	Anomocytic	00	14.86	00	2189	00	0.353	00	0.057	00	0.032
9	Psidium gaujava.L	Anomocytic	14.06	23.43	1791	5971	0.203	0.259	0.145	0.172	0.047	0.070
10	Ipomoea sp.	Paracytic	12.5	16.98	796	1791	0.428	0.389	0.143	0.174	0.097	0.107
11	Tinospora cordifolia (Thunb.) Miers	Anomocytic	00	10.63	00	995	00	0.561	00	0.253	00	0.227

Table 1.1 – Comparison of stomatal index

List Names	Number of elements	Number of unique elements
Upper	11	9
Lower	11	11
Overall number of unique elements		19

Table 1.2

Names	Total	Elements
Upper	1	00
Upper, Lower	1	12.5
Upper	7	6.66 14.06 28.26 10.81 14.28 18.51 13.54
Lower	10	16.98 6.59 17.5 23.43 17.91 15.91 10.63 21.57 26.92 14.86

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Table 1.3 – Comparison of stomatal frequency

List Names	Number of elements	Number of unique elements
Upper	11	7
Lower	11	9
Overall number of unique elements		14

Table 1.4

Names	Total	Elements
Upper	1	00
Upper/Lower	2	1990 1791
Upper	4	398 2588 796 1592
Lower	7	4578 2389 1194 5971 2189 995 2787

Table 1.5 – Comparison of length, breadth and area of stomatal aperture (Upper epidermis)

List Names	Number of elements	Number of unique elements
Length	11	9
Breadth	11	9
Area	11	9
Overall number of unique elements		25

Table 1.6

Names	Total	Elements
Length, Breadth, Area	1	00
Length	8	0.366 0.203 0.399 0.299 0.292 0.269 0.428 0.268
Breadth	8	0.042 0.134 0.050 0.094 0.048 0.145 0.068 0.143
Area	8	0.045 0.047 0.097 0.082 0.023 0.031 0.041 0.021

Table 1.7 – Comparison of length, breadth and area of stomatal aperture (Lower epidermis)

List Names	Number of elements	Number of unique elements
Length	11	11
Breadth	11	11
Area	11	11
Overall number of unique elements		33

Table 1.8

Names	Total	Elements
Length	11	0.184 0.234 0.247 0.272 0.389 0.561 0.348 0.298 0.353 0.371 0.259
Breadth	11	0.174 0.027 0.053 0.129 0.078 0.182 0.074 0.102 0.172 0.057 0.253
Area	11	0.035 0.012 0.107 0.032 0.015 0.029 0.227 0.040 0.070 0.071 0.108

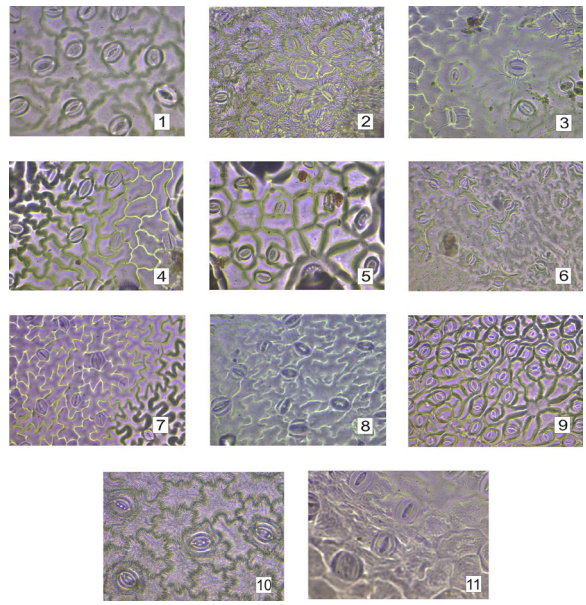

Plate 1: Microscopic photographs (40X) of various stomatal types in leaves of wild plants. 1. Plumbago zeylanica L showing anisocytic stomata. 2. Sida acuta Burm.f showing paracytic stomata. 3. Alternanthera pungens Kunth showing paracytic stomata. 4. Conyza stricta Less showing anomocytic stomata. 5. Achyranthes aspera L showing anomocytic stomata. 6. Pongamia pinnata.L showing anomocytic stomata. 7. Amaranthus viridis.L showing anisocytic stomata. 8. Ficus racemosa.L showing anomocytic stomata.9. Psidium gaujava.L showing anomocytic stomata. 10. Ipomoea sp. showing paracytic stomata. 11. Tinospora cordifolia (Thunb.) Miers showing anomocytic stomata.

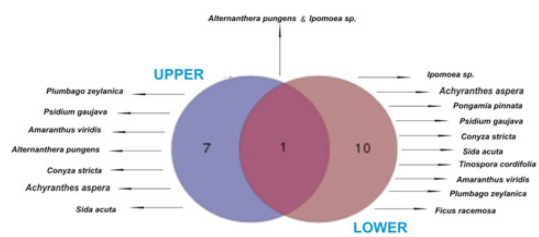

Figure 1: Comparison of stomatal index

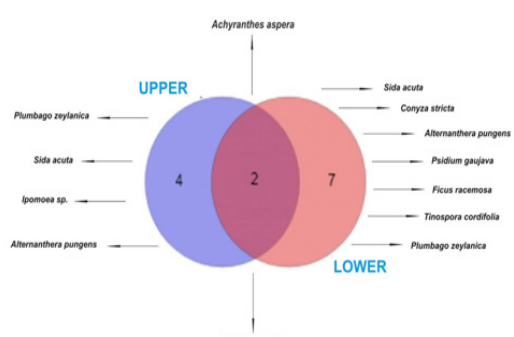

Figure 2: Comparison of stomatal frequency

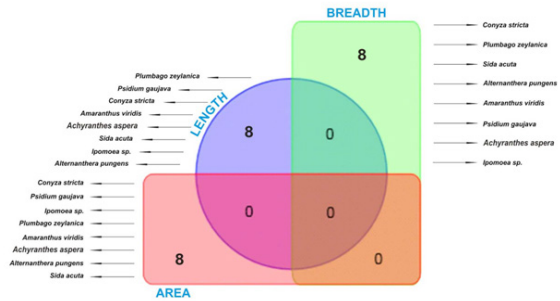

Figure 3: Comparison of length, breadth and area of stomatal pore. (Upper epidermis)

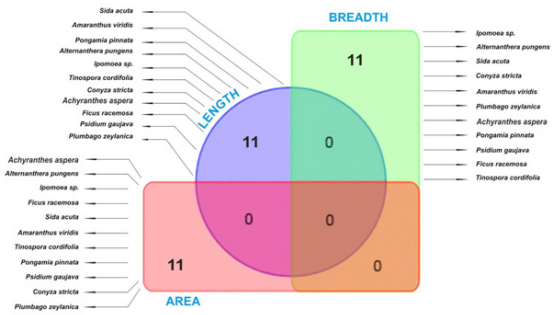

Figure 4: Comparison of length, breadth and area of stomatal pore. (Lower epidermis)

Conclusion

From the above results, it can be stated that, variation in each stomatal parameter differs from one plant species to another. As comprehended by Essiett et al. (2012), the presence and combination of different types of stomata on the surface of leaves can be useful in the process of classification. And the stomatal index is independent of the environment, size or portion of the leaf surface, size of the intervening epidermal cell.

Stomatal index and the other studied parameters are highly constant for any given species. By this analysis, substantial differences in the stomatal parameter of several wild plants were uncovered. The ability of these several wild plants to survive in a unique environmental region is also well marked.

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REFERENCE

AbdulRahaman, A.A. & Oladele, F. A. (2003). Stomatal complex types, stomatal size, density, and index in some vegetable species in Nigeria. Nigerian Journal of Botany, Volume 16, 144 – 150. | Belhadj, Safia., Derridj, Arezki., Moriana, Alfonso., Gijon, Maria Del Carmen., Mevy, Jean-Phillipe., and Gauquelin, Thierry. (2011). Comparative analysis of stomatal characters in eight wild atlas pistachio populations (Pistacia atlantica Desf.; Anacardiaceae). International Research Journal of Plant Science , Volume 2(3), 060-069. | | Chen, Li-Qun., Sen Li, Cheng., Chaloner G, William., Beerling, David J., Sun, Qi-Gao., Collinson, Margaret E., & Mitchell, Peter L. (2001). Assesing the potential for the stomatal characters of extant and fossil Ginko leaves to signal atmospheric CO2 change. American Journal of Botany, Volume 88(7): 1309–1315. | | Croxdale, Judith, L. (2000). Stomatal patterning in angiosperms. American Journal of Botany, Volume 87(8), 1069–1080. | Dalal, L.P. (2012). Stomatal variation in Dicot and Monocots - A Case Study. Asiatic Journal of Biotechnology Resources, Volume 03 (10), 1473-1477. | Essiett, U. A., Illoh, H. C., and Udoh, U. E. (2012). Leaf epidermal studies of three species of Euphorbia in Akwa Ibom State. Advances in Applied Science Research, Volume 3 (4), 2481-2491. | Hetherington, Alistair M. & Woodward, F. Ian. (2003). The role of stomata in sensing and driving environmental change. Nature Publishing Group, Volume 424, 901 – 908. | | Metcalfe, C.R. & Chalk, L. (1950). Anatomy of the Dicotyledons, | Vols.I and II. Clarendon Press, Oxford. | | Perveen, Anjum., Abid, Rubina., & Fatima, Rabab. (2007). Pakistan Journal of Botany, Volume 39(4), 1017-1023. | Salisbury EJ. (1927). On the causes and ecological significance of stomatal frequency, with special reference to woodland flora. Philosophical Transactions of the Royal Society London, Volume B216, 1-65. |