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RELATIONSHIP BETWEEN THE LEAF AREA AND TAXONOMIC IMPORTANCE OF FOLIAR STOMATA

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ABSTRACT

The stomata are apertures in the epidermis, each bounded by two guard cells. Their main function is to allow gases such as carbon dioxide, water vapours and oxygen to move rapidly into and out of the leaf. In green leaves they occur either on both surfaces (amphistomatic leaf) or on one only, either the upper (epistomatic leaf) or more commonly the lower i.e., hypostomatic leaf. Four types of stomata are recognized such as anomocytic, paracytic, diacytic, and anisocytic from the materials under study such as *Manihot esculenta* Crantz, *Colocasia esculenta* (L.) Schott, *Maranta arundinacea* L., *Annona squamosa* L., *Artocarpus heterophyllus* Lam., *Passiflora edulis* Sims., *Curcuma longa* L., *Mangifera indica* L. and *Garcinia cambogia* (Gaertn.) Desr. Among all of the stomatal types paracytic type is dominated. The guard cell of *Colocasia esculenta* is kidney or bean shaped. But in other monocotyledonous plants under the present study *Curcuma longa* and *Maranta arundinacea* are with dumbbell shaped guard cell. The aim of the present study is to correlate between foliar characters and stomata in leaf.

KEYWORDS: Stomata, Anisocytic, Paracytic, Diacytic, and Foliar Characters

INTRODUCTION

Stomata (singular, stoma), is acting as a connection between the internal air spaces of plants and the external atmosphere. The external surfaces of most herbaceous plants and the leaves of woody plants are covered with a compact waxy layer which is not only impermeable to water vapour but also enables plants to conserve water in dry air. This thick waxy layer of cuticle hampers the entrance of the carbon dioxide essential for carbon fixation in photosynthesis. Stomata are pores in the epidermis and associated cuticle bordered by pairs of structurally and physiologically specialized guard cells and adjacent epidermal cells termed subsidiary cells. This group of cells forms the stomatal complex and facilitates gas movement through the epidermis.

In green leaves stomata occur either on both surfaces, amphistomatous or on one surface. If the stoma is present only on the upper epidermis the leaves are called as epistomatous and if it is on the lower epidermis the leaves are called as hypostomatous. On the other hand a leaf with stomata only on the adaxial (upper) surface (epistomatous) is found in some floating plants, such as water lilies (Lawson, 2009).

Two distinct types of guard cells exist in leaves, kidney shaped and dumb-bell shaped. Kidney-shaped guard cells are found in dicotyledons whereas dumb-bell-shaped guard cells are found in grasses. Dumb-bell shaped guard cells are more advanced in evolutionary terms and more efficient physiologically because guard cells of grasses require fewer solutes and less water to achieve a given unit increase in aperture. Directly beneath each pair of guard cells inside the leaf

is a substomatal cavity. Air in this cavity in living leaves is virtually saturated with water vapour because of evaporation from adjacent wet cell walls (Brian et al., 1999)

The presence of pores in leaves was envisioned by Malphiji in 1674 and. de Candolle in 1872 has given the term "stomata" was proposed to these pores. Grew in1682 (Jarvis and Mansfield, 1981). Stomata occur on stems, leaves, flowers, and fruits, but not on aerial roots and vary widely in size and frequency (Smith et al., 1989). In monocots, conifers, and some dicots, stomata occur in parallel rows, but in leaves with netted venation they are scattered. They sometimes are sunken below the surface but occasionally are raised, and usually they open into substomatal cavities in the mesophyll tissue. They are easily visible on leaf surfaces under magnification because of the peculiar shape of the guard cells and the fact that guard cells, unlike other epidermal cells, usually contain chloroplasts (Kramer and Boyer, 1995).

The early history of stomatal nomenclature dates back to Prantle in 1872 (Jarvis and Mansfield, 1981). Francey (1936) recognised eight categories and 34 structural stomatal types based on the number, position and size of the subsidiaries. On the basis of arrangement of the epidermal cell neighbouring the guard cell, more than 25 main types of stomata in dicots have been recognized.

Stomatal density can vary within leaves, plants, and individuals of a single species (Al Afas, 2006). It can also vary due to environmental factors such as light, air humidity, water availability and atmospheric CO_2 concentration (Woodward and Kelly, 1995). In general, stomatal density decreases with increase in CO_2 and in comparison with leaves developed under low light intensity, sun exposed leaves have higher stomatal densities (Givinish, 1998). In amphistomatous leaves, stomatal frequency is usually greater in the lower leaf surface than in the adaxial side, and the adaxial to total stomatal density ratio tends to decline with decreasing irradiance (James and Bell, 2000). In trees, stomatal density often ranges from 30 to 1190 mm^{-2} and guard cell length from 10 to 50 µm, and often a negative relationship between stomatal density and stomatal size has been observed (Hetherington and Bell, 2000). The present study is aimed to find out a relationship between the leaf area, stomatal size and stomatal density.

METHODOLOGY

Collection of Specimens

The present work is done in 2014 at Sree Narayana College Nattika, Thrissur, Kerala. The specimens were collected from the Sree Narayana College, Nattika, Thrissur. Five leaves of each of the study materials were used for this work. It includes:

- **Cassava:** *Manihot esculenta* Crantz
- **Taro:** *Colocasia esculenta* (L.) Schott
- **Arrow Root:** *Maranta arundinacea* L.
- **Sugar Apple:** *Annona squamosa* L.
- **Jack Fruit:** *Artocarpus heterophyllus* Lam.
- **Passion Fruit:** *Passiflora edulis* Sims.

- **Turmeric:** *Curcuma longa* L.
- **Mango Tree:** *Mangifera indica* L.
- *Garcinia cambogia* (Gaertn.) Desr.

Four parameters involving stomatal studies were investigated in this study; namely,

- The number of stomata per unit area of leaf surface,
- The size of the stomata as judged by
- The length of the stomatal slit or opening,
- Type of stomata.

Isolation of Leaf Epidermal Layers

Epidermis of leaf is isolated from fresh plant specimen. The mature leaves were fixed in FAA solution (acetic acid: alcohol: formalin: water = 2:5:1:12) for 24 hours and washed in 70% ethanol. Three circular disk samples were cut from an area adjacent to the midrib of each leaf. Disk sample was boiled in 5% aqueous solution of KOH for 5-10 minute. The upper (adaxial) and lower (abaxial) surfaces were separated with dissecting needle and forceps and rinsed with clean water. In the present study only the abaxial epidermis is used.

Staining, Mounting and Observation of Leaf Surfaces

Epidermal peals were stripped and stained with 1% (w/v) in 50% aqueous ethanol, saffranin and temporary mount in glycerine. Excess stain was rinsed off with clean water and mounted in glycerine. Observations were made on the microscope to determine, stomatal complex types and their frequencies, stomatal size, stomatal density and stomatal index.

Photomicrography

After preparing, slides were observed under light microscope [(40x) Magnus DM 1000] for detailed analysis and obtaining better picture as well as measuring the length and breadth of stomata including guard cells.

Determination of Leaf Area, Stomatal Density and Index

The leaf area is calculated using a graph paper.

- The leaf to be measured is laid on graph paper that has 1 cm^2 grid lines.
- The outline of the leaf is traced using a pencil.
- The number of grid squares that are inside of the leaf outline on the paper is counted.
- The number of boxes is added up.

Stomatal Index

The stomatal density or stomatal index or stomatal frequency was based on average obtained from observations of 3 microscopic fields. Stomatal index was calculated according to the following method of Meidner and Mansfield (1968).

Determination of Stomatal Size

The mean stomatal size or area of a species was determined by measuring length of guard cell using an eye-piece micrometer.

RESULTS AND DISCUSSIONS

The results in this investigation were summarised in tables numbered 1, 2 and 3 including stomata count, stomata type, stomatal index, stomatal size and leaf area.

The fundamental purpose of leaves is harvesting light. Under full-sun conditions, the photosynthetic capacity per unit leaf area increases, this resulting in increased thickness in leaves, accompanied by the production of an additional layer of palisade cells. Nevertheless, in the present study the effect of light conditions on mesophyll thickness was not observed. It is generally assumed that shade leaves are thin and large, having a lower SD than that found in sun-exposed leaves (Fahn, 1990). The shape of foliar epidermis is one of the significant taxonomic characters. Taxonomic studies of a number of families are based on leaf epidermis anatomy (Bhatia, 1984).

***Manihot esculenta* Crantz**

Present study showed Undulating to irregular epidermal cells of the leaves and the stomata is paracytic type (Plate 1 to 3). Cerqueira (1989) also reported that the stoma in cassava is paracytic type. The leaf showed stomatal index 33.3% and are with 40 stomata per square centimetre. The size of the leaf is 85.2 cm^2. The stomatal size is 92.4 µm.

***Colocasia esculenta* (L.) Schott**

Colocasia esculenta leaves are with pentagonal epidermal cell and the guard cell stomata is kidney or bean shaped (Plate 1 to 3). The type of stomata is paracytic. Stomatal index is 55.2% and are with the stomatal count 72 per cm^2. According to Saadu *et al* (2009) C. esculenta and C. hortulanum have brachy-paracytic stomatal complex type with a frequency of 100%. The size of the leaf is 150.8 cm^2. The stomatal size is 16.2 µm.

***Maranta arundinacea* L.**

In the present study *Maranta arundinacea* variously shaped epidermal and the stomata is diacytic type (Plate 1 to 3). The leaf showed stomatal index 19.1% and are with 12 stomata per square centimetre. (Tomilson, 2008). The size of the leaf is 109 cm^2. The stomatal size is 97.53 µm.

***Annona squamosa* L.**

Annona squamosa leaves are with pentagonal epidermal cell and the guard cell stomata is kidney or bean shaped (Plate 1 to 3). The type of stomata is anomocytic. Stomatal index is 22.2% and are with the stomatal count 8 per square centimetre. The size of the leaf is 51 cm^2. The stomatal size is 71.8 µm.

***Artocarpus heterophyllus* Lam**.

In the present study *Artocarpus heterophyllus* leaves are with undulating to irregular shaped epidermal cells and the stomata are paracytic type (Plate 1 to 3). The leaf showed stomatal index 12.5% and are with 15 stomata cm^2. The size of the leaf is 93cm^2. The stomatal size is 66.7 µm.

***Passiflora edulis* Sims**

Stomata on leaves are arranged at random directions. The leaf of passiflora shows both anisocytic and paracytic type of stomata (Plate 1 to 3). Paracytic stomata is the most commonly seen type. Anisocytic are fewer in number. Kyoichi (1967) reported that the size and type of stoma varies according to the organ or part on which stomata exist. stomata on lamina and epicarp are arranged at random directions, stomata are arranged paralleling to longitudinal are arranged paralleling to vascular bundle. The leaf showed stomatal index 38.2% and are with 22 stomata per square centimetre. The size of the leaf is 114 cm^2. The stomatal size is 102.6 µm.

***Curcuma longa* L.**

The epidermis consists of pentagonal cells that are predominantly elongated at right angle to long axis of the leaf (Plate 1 to 3). Stoma is paracytic. The present result is in contradiction with the studies of Ravindran *et al* (2007). They reported that the stomata are diperigenous to tetraperigenous and in some cases they are anisocytic. The leaf showed stomatal index 10.6% and are with 12 stomata per square centimetre. The size of the leaf is 120 cm^2. The stomatal size is 169.4 µm.

***Mangifera indica* L.**

Norfaizal *et al* (2013) reported that Anomocytic, anisocytic and staurocytic stomata were observed in Mangifera (Plate 1 to 3). In the present study the epidermis is with round or oval shaped cells and the stoma is anisocytic type. The leaf showed stomatal index 30% and are with 46 stomata per square centimetre. The size of the leaf is 63.4 cm^2. The stomatal size is 35.9 µm. Urban and Jannoyer (2004) reported that the first striking feature of mango seems to be well protected against excessive losses of water through transpiration. Stomatal frequency is high but stomata are small, which allows for quick responses to relevant stimuli. Transpiration curves show that a loss of less than 2% of water can lead to complete stomatal closure.

***Garcinia cambogia* (Gaertn.) Desr.**

In the present study epidermal cells are uniseriate and comprise of undulating to irregular cells (Plate 1 to 3). The outer cell walls of the epidermal cells are well cuticularised with cuticular ridges. The stomata is paracytic. This result is in accordance with Pathirana and Herat (2004). The leaf showed stomatal index 10% and are with 8 stomata per cm^2. The size of the leaf is 60 cm^2. The stomatal size is 107.8 µm.

The plants selected are growing under full-sun conditions. Among the plants *Colocasia esculenta* showed highest stomatal index and stomatal density. *Manihot esculenta,* and *Passiflora edulis* comes next with stomatal index and stomatal density 33.3%, 14 and 38.2% and 13. When compared with area *Manihot esculenta* and *Colocasia esculenta* attains highest position between the materials understudy followed by *Maranta arundinacea* and *Curcuma longa.*

In the present study *Colocasia esculenta* is with largest leaf area but it shows the highest stomatal frequency and least stomatal width. Earlier study by Metcalfe and Chalk (1988) showed that large stomata resulted in low stomatal density while small stomata gave high stomatal density. The work of Abdul Rahaman and Oladele (2003) also showed this pattern where, large stomata actually gave low stomatal density and small stomata gave high density in some vegetable species.

Some correlations do occur between the stomatal features (like density, index and size) and rate of transpiration in each species studied. Stomatal density has been identified to play major role in water use efficiency of plants thus, its numerical strength on the leaf surface is essential (Givnish, 1988). High stomatal density, high index and large stomata give high transpiration rate. In the materials under study *Curcuma longa* and *Garcinia cambogia* are with lowest stomatal density and higher stomatal width. This property is to reduce the transpiration rate of the plants. So the rate of transpiration will be lower in *Curcuma longa* and *Garcinia cambogia* when compared with other materials under study.

Nature of Guard Cell

Colocasia esculenta the guard cell of the stomata is kidney or bean shaped. But in other monocotyledonous plants under the present study *Curcuma longa* and *Maranta arundinacea* are with dumbbell shaped guard cell. According to Brian *et al* (1999) dumbbell shaped guard cells require very less amount of solute and water for opening and closing of guard cell. Thus the grasses can easily control the opening and closing of the stomata and adjust with water deficiency and will reduce transpiration.

Low transpiration rates of C4 annuals might be explained with comparably small rooting systems limiting the water uptake under high pressure gradients. The C3 annual dicotyledonous plant, however follows an opportunistic strategy with high water consumption. Plants photosynthetic performance combined with morphological and functional traits of leaves are promising indications of adaptation mechanisms of plant species under semi-arid conditions (Dong et al., 2012).

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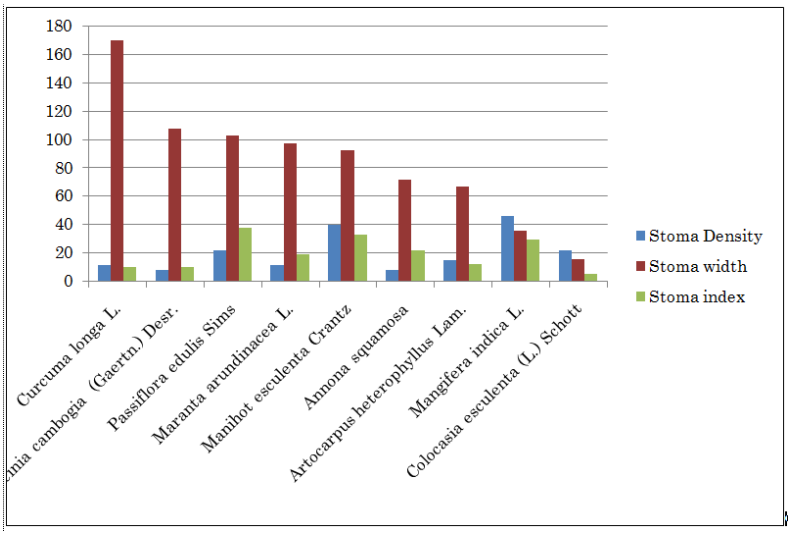

Figure 1: Relationship between Stomatal Density, Width and Index

Table 1: Relationship between Stomatal Characters under Study

Sl. No	Taxa	Habit	Group	Surface	Leaf Epidermis Cell Shape	Stomata				
						Presence of Stomata	Stomatal Types			
							Anomocytic	Anisocytic	Paracytic	Diacytic
1	Manihot esculenta Crantz	Perennial Woody shrub	Dicot	Abaxial	Undulating to irregular	+	-	-	+	-
2	*Colocasia esculenta* (L.) Schott	Perennial Herb	Monocot	Abaxial	Pentagonal	+	-	-	+	-
3	*Maranta arundinacea* L.	Perennial Herb	Monocot	Abaxial	Variously shaped	+	-	-	-	+
4	*Annona squamosa* L.	Shrub	Dicot	Abaxial	Variously shaped	+	+	-	-	-
5	*Artocarpus heterophyllus* Lam.	Tree	Dicot	Abaxial	Undulating to irregular	+	-	-	+	-
6	*Passiflora edulis* Sims	Woody perennial vine	Dicot	Abaxial	Variously shaped	+	-	+	+	-
7	*Curcuma longa* L.	Perennial Herb	Monocot	Abaxial	Pentagonal	+	-	-	+	-
8	*Mangifera indica* L.	Tree	Dicot	Abaxial	Round/Oval	+	-	+	-	-
9	*Garcinia cambogia* (Gaertn.) Desr.	Tree	Dicot	Abaxial	Undulating to irregular	+	-	-	+	-

Table 2: Relationship between Stomatal Characters under Study

Sl. No.	Taxa	Habit	Stomatal Index Percentage	Stomatal Count/cm^2
1	*Manihot esculenta* Crantz	Perennial Woody shrub	33.3 %	40
2	*Colocasia esculenta* (L.) Schott	Perennial Herb	55.2 %	72
3	*Maranta arundinacea* L.	Perennial Herb	19.1 %	12
4	*Annona squamosa* L.	Shrub	22.2 %	8
5	*Artocarpus heterophyllus* Lam.	Tree	12.5 %	15
6	*Passiflora edulis* Sims	Woody perennial vine	38.2 %	22
7	*Curcuma longa* L.	Perennial Herb	10.6 %	12
8	*Mangifera indica* L.	Tree	30 %	46
9	*Garcinia cambogia* (Gaertn.) Desr.	Tree	10 %	8

Table 3: Relationship between Leaf Area and Width of Stomata

Sl. No.	Taxa	Leaf Area	Width of Stomata
1	*Manihot esculenta* Crantz	85.2	92.4
2	*Colocasia esculenta* (L.) Schott	150.8	16.2
3	*Maranta arundinacea* L.	109	97.53
4	*Annona squamosa* L.	51	71.8
5	*Artocarpus heterophyllus* Lam.	93	66.7
6	*Passiflora edulis* Sims	114	102.6
7	*Curcuma longa* L.	120	169.4
8	*Mangifera indica* L.	63.4	35.9
9	*Garcinia cambogia* (Gaertn.) Desr.	60	107.8