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Geometry from stomata networks at leaves of the *Ctenanthe oppenheimiana*

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Abstract. We measure the stomatic plasticity at *Ctenanthe oppenheimiana* leaves based on distance between stomata pairs in microscopic images. Theoretical graphs were built according with distance thresholds as connectivity parameter. The theoretical networks based at distances between stomata depends on how the connectivity can vary according with the threshold distances. Plants exposed to extreme light irradiation times from 24 to 4 hours per day, presented discernible relations between stomatic distances. The graphs related to average distances between stomata pairs revealed a powerful tool to predict changes of the geometric distribution of stomata at live *Ctenanthe oppenheimiana* plants.

1. Introduction

Stomata are cells that act as valves localized at plant leaves and stems performing the exchange of gas with the atmosphere [1]. They control the relation between the carbon gain and water loss to adjust photosynthetic needs with environmental and atmospheric conditions [2]. The plant epidermis is highly plastic during environmental adaptation, thus the number of stomata per leaf area can be highly variable and adapted according to the environmental conditions. [3]. Many works have have measured of the distribution of stomata from the epidermis as parameter to build theoretical graphs, based on distance intervals between pairs of stomates at the plant epidermis [4].

Therefore the distribution of stomata in the epidermis can be a very valuable quantitative parameter in the analysis of plasticity as it provides many details of distance that can change at plant adaptation to certain environmental conditions.

In this study, we attempt to develop computational metrics based on distance between stomata pairs located in the microscopic images from the extant *Ctenanthe oppenheimiana* plants using different conditions of acclimatization to light as environmental change, and developed theoretical graphs as a tool to further characterization of the stomatic networks based on distance between stomata pairs.

2. Methods

2.1. Optic microscopy and plant acclimation

We performed optic microscopy with *Ctenanthe oppenheimiana* leaves from plants artificially adapted at controlled growth chambers. The microscope used was Axio-Lab A1, Zeiss, adapted



with cameras axiocam ERc5s, the magnification used was $100\times$. The plants used at microscopy were adapted with continuous 55% relative humidity, photon flux from $100\mu\text{mol}/\text{m}^2 \times s$, temperature 25°C and light photo periods from 24 and 4 hours per day during a period of 60 days.

2.2. Model to build graphs with stomata from microscopic images

For each microscopic image a graph with stomata over the leaf can be modeled. The segmented stomata (s_i) from the leaf image represents a node in the graph. The criterion for establishing the edges is based on the distance between pairs of stomata, where a threshold radius (T_r) determines whether two stomata get connected with each other. The threshold parameter controls the connectivity of the network. For instance, as T_r increases, more connections will be established between the centroids of stomata, and therefore, the density of the network will be higher [4]. Moreover, note that, through this threshold criterion, a great number of networks can be built by modifying the range condition, which allows to analyze several independent networks.

A stomatic network is defined by the adjacency matrix A , which receives values of zero or ones, whether the distance values between pair of stomata $d(s_i, s_j)$ are within the limited range T_r as follows:

$$A_{i,j} = 1, \text{ if } d(s_i, s_j) \leq T_r \\ 0, \text{ otherwise}$$

The construction of a stomata network from microscopic images is illustrated in Figure 1.

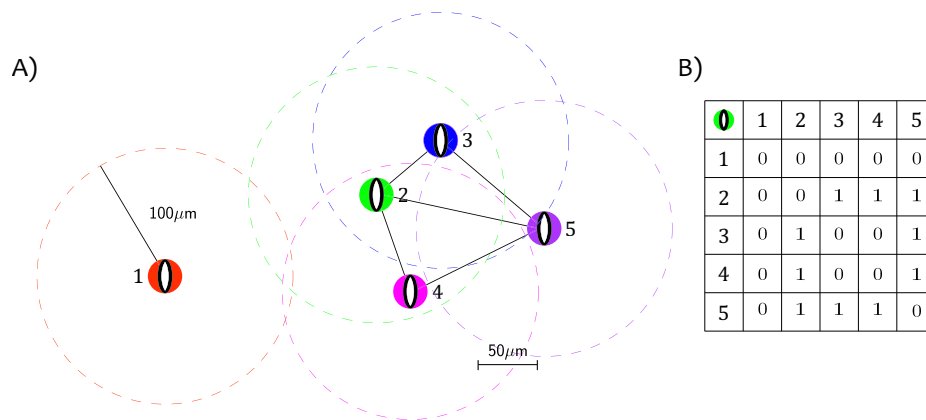


Figure 1. Model of a stomatal network. A) The stomata are delimited by a circumference with a given threshold radius of $T_r = 100 \mu\text{m}$. If the distance between stomata pairs is less than or equal to $100\mu\text{m}$, then an edge (filled line) is established between them. Observe that each stomata is at the center of a circumference depicted with same color, which defines the connection radius from one stomata with each other on the network. B) Corresponding adjacency matrix of the stomatic network depicted in A).

2.3. Network parameters

Several measures that characterize the statistics and topology associated with the stomatic network can be calculated as soon as an adjacency matrix of each image is established. The number of stomata per sample is represented by the number of nodes (N), and the number of edges (E) refers to all possible stomata present inside the threshold radius (T_r). The degree or

connectivity k_i of a stomata s_i is defined as the number of neighbors that s_i posses, which is given by

$$k_i = \sum_{j=1}^N A_{ij}. \quad (1)$$

The connectivity is also usually measured in global terms as

$$\langle k \rangle = \frac{1}{N} \sum_{i=1}^N \sum_{j=1}^N A_{ij}. \quad (2)$$

that is the mean degree to all stomata of the network in each microscopic image.

3. Results

3.1. Images from extant leaves

Microscopic images collected from fresh leaves of the plant showed an interesting pattern of stomata completely visible on the abaxial epidermis (figure 2). It revealed a contrast between the stomata of the epidermis (green) and the pavement cells (purple), with greenish cellular walls between the pavement cells.

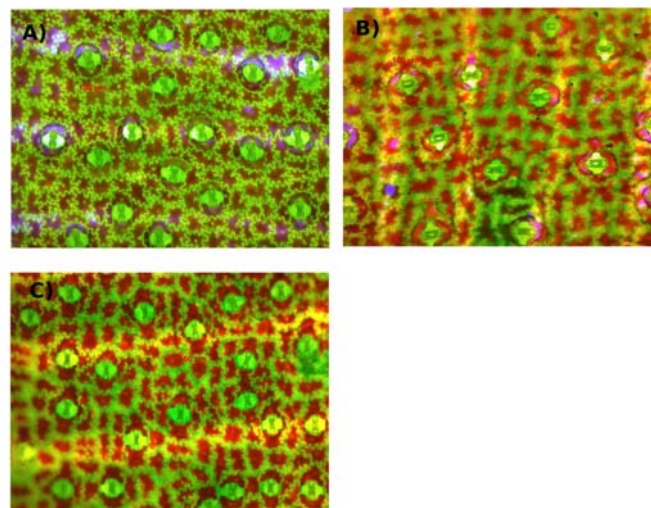


Figure 2. Microscopic images of the abaxial epidermis from plant submitted to photo periods of 24 and 4 hours per day with artificial illumination and solar photo periods. Magnification of 200X. A) Plant exposed to photo period of the 24 hours per day. B) Plant exposed to photo period of the 4 hours per day. C) Plant exposed to solar photo periods. Size scales in red

3.2. Stomatic networks

Distance thresholds (T_r) were used as a condition to construct the graphs, in agreement with what is described in the methods (figure 1). The network connectivity depend on the distance between the stomates in the images and the distances vary according to the stomatic densities. Based on it the network connectivity can be compared according with the thresholds adopted at network percolation.

The number of edges added in the networks of plants exposed to 24h of daily illumination is proportionally greater than in the plants exposed to 4 hours.

The figure below (figure 3) illustrates the relation between the average values of the mean degree $\langle\langle k \rangle\rangle$ in function of network percolation at several distance thresholds (T_r) in 18 plants exposed to 4 and 24 hours of daily illumination.

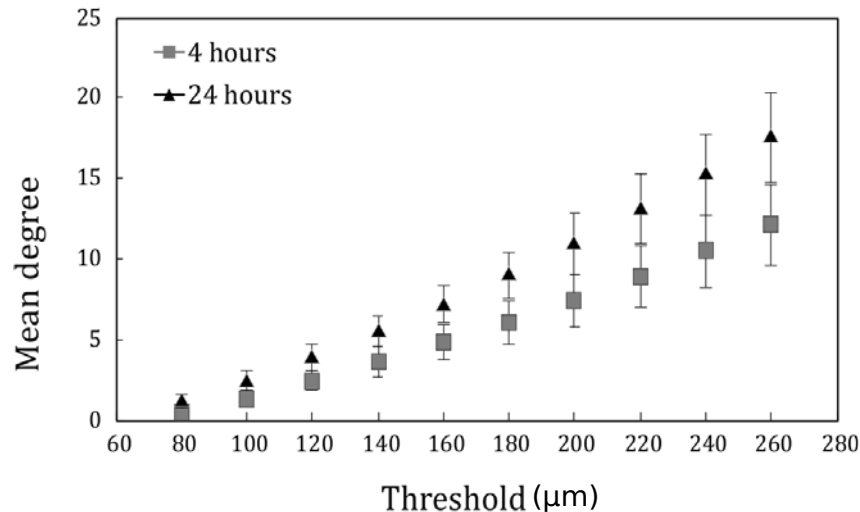


Figure 3. Dispersion of the the mean values of the mean degree $\langle\langle k \rangle\rangle$ in 10 distance thresholds in plants submitted to 4 (grayish square), and 24 hours (dark triangle) of daily illumination, the error bars shows the range of variation relative to the mean values of images of the 18 plants.

4. Conclusions

We showed here that the stomata distribution over the leaf surfaces (*Ctenanthe oppenheimiana*) varies according with the light acclimatization conditions. The quantification of changes at the stomata plasticity over the leaves can be performed by means of graphs based at distances between stomata. Therefore the precise measures of the graph properties at stomatic networks, specially at the *Ctenanthe oppenheimiana*, can be useful as a sensor to environmental changes that plants can be exposed in general.

5. Acknowledgements

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6. References

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