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Stomatal Complex and transpiration rates in some members of Rutaceae and Myrtaceae

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Abstract

Stomata complex and transpiration rates in *Eucalyptus torelliana, E. camaldulensis* (Myrtaceae)*; Citrus limon and C. sinensis* (Rubiaceae) were studied, using standard methods. All plant species used in this experiment were collected within the Campus of Kogi State University. The leaves of Eucalyptus species were amphistomatic while those of Citrus species, hypostomatic. The stomatal complex types in the species studied are quite heterogenous. Seven stomata complex types were recognized in all the species studied namely; anisocytic, anomocytic, tetracytic, paracytic, actinocytic, cyclocytic and pericytic. *Eucalyptus camaldulensis* with five types of stomata complex has the highest transpiration rate (3.6063 x 10-3mol/m2/sec.), followed by *E. torreliana* with four stomata complex types (3.1522 x 10-3mol/m2/sec.). *Citrus sinensis* with three stomata types (1.7992 x 10-5mol/m2/sec.). *C. limon* with the two stomata types had the least transpiration rate (1.2503 x 10-5mol/m2/sec.). Variances in density and location of stomata complex types is correlated with transpiration rate and the implications of these are briefly discussed.

INTRODUCTION

The Rutaceae family has about 162 genera and 1650 species, distributed in warm temperate and tropical regions with the greatest diversity in Australia and South Africa. They are usually trees or shrubs in habits. The family is important for its fruits, ornamental shrubs and medicinal uses [1]. Myrtaceae family has about 144 genera and 3000 species. They are mainly distributed in tropics and subtropics, abundant in Australia. They are evergreen shrubs or large trees which provide a good source of important oils [1]. In plants, water in form of vapor gets lost to the atmosphere by transpiration via stomata in the leaf surface. Plants conserve soil water by encouraging percolation and discouraging surface runoff. This water influences the atmospheric humidity. Transpiration usually account for about 99% of water uptake by plants. Thus, only 1% of water taken up by the plants is used in metabolic activities. Stomata density has been identified to play major role in water use efficiency of plants thus, its numerical strength on the leaf surface is essential [2, 3]. High stomata density has been associated with high humidification potential, vice versa [4, 5].Other factors like the potassium ion concentration, the pH, sugar concentration, light, carbon (iv) oxide concentration and saturated water vapor pressure deficit [6, 7]; activities of abscisic acid, internal leaf water status, hydraulic properties and water potential of the vascular system as well as the availability of water in the soil and atmosphere [8, 9] also have considerable effects on transpiration rate in plants.Whereas certain stomata types are diagnostic of certain families, several families and species exhibit multiple stomata complex types [4,5,10,11]. [11,12] have previously recognized two stomata types in some members of Myrtaceae and four in Rubiaceae respectively.

In recent time, leaf epidermal characters have received very considerable attention by taxonomists [13]. Epidermal features became widely studied from the ontogenetic, phylogenetic and taxonomic perspectives. Ontogenetic investigations have tended to show methods of origin of the different stomata types resulting to classification of stomata based on their ontogeny. Phylogenetic considerations make use of the presumed relationships between the different patterns of stomatal ontogeny in proposing evolutionary pathways between taxa while taxonomic considerations make use of epidermal features for identification, classification and naming taxa [11].

The use of epidermal characters such as stomata types, trichome types, stomatal frequency and index in classification seems to be increasing rapidly because not only do epidermal characters correlate with gross morphological features in most cases, they are often known to be very valuable at the levels where classical methods of cytological and genetics cannot be applied [14]. Many workers showed that leaves posses many morphological attributes of potential taxonomic significance that are often diagnostic at the genus and species levels [15, 16, 17]. The shape of epidermal cells, types and arrangement of stomata and size and shape of trichomes are important systematic parameters. [16] studied the leaf epidermal features of *Vigna* species and separate *V. gracillis* and *V. racemosa* from other species based on trichome morphology.The experiment was designed to investigate the effects of the stomata complex types on transpiration rates in the two families.

MATERIALS AND METHODS

All samples for the study were collected from Kogi State University, Anyigba, Nigeria. The identification was done by the curator at the Department of Biological Sciences, Faculty of Natural Sciences, Kogi State University, Anyigba. Voucher herbarium specimens were deposited in the University Herbarium.

Determination of Transpiration rate

Cobalt chloride paper method was used to determine the transpiration rate of each specimen [4, 5, 12, 18]. 10g of cobalt chloride anhydrous was dissolved in 200 ml of distilled water. The 200 ml of cobalt chloride solution was divided into four beakers each containing 50 ml. Strips of filter paper of 2×6cm dimension were cut. 20 strips were immersed in each of the beaker and allowed to stand for 24 hours. After 24 hours, the strips were dried thoroughly in an oven at 100°C for 20 minutes to obtain a deep

blue coloration. The dried strips were kept in a dessicator. For each species studied, three of the dried strips were placed in sealed air tight polythene bags and weighed (W_1: initial weight), using an electronic weighing balance. It was transferred quickly to the field and affixed with a string (thread) to the marked branches of each tree with leaves on the abaxial surface. The time taken for the strips to turn pink was noted in seconds for each case. Once the strip turned pink, the polythene bag was quickly untied and sealed again, transferred to the laboratory and weighed (W_2: final weight). The procedure was used was replicated ten times for all the species. The amount of water transpired was determined as W_2-W_1. The surface area of the leaves were determined using graph sheets. The amount of water transpired (W) in grams were converted to mole by the formula:

$$\text{Mole} = \frac{\text{Amount of water transpired (W)g}}{\text{Molar mass of water g/mol}}$$

Transpiration rate was expressed as water loss in $mol/m^{-2}/s^{-1}$.

Isolation of Epidermal layers

Fresh matured leaves of the four species were collected, washed in clean water and allowed to dry. After drying, the leaves were painted with nail varnish on both surfaces (i.e. abaxial and adaxial) and allowed to dry. After drying, a short clean cellophane tape was firmly pressed over the dry surface. The tape was carefully peeled from the leaf. The obtained cuticular peel was stained in 10% safranin for 10 minutes and mounted in glycerol on a clean slide and covered with clean cover slip for microscopic study.

Determination of stomatal types, size, density density of epidermal cells.

Using an Olympus microscope at x40 objective, 30 fields of view of each sample were studied to determine the types of stomata complex; stomata size (product of the length and breadth of the guard cells); stomatal density (no. of stomata per mm^2). Means of the data on the parameters of the anatomy of leaf epidermis were subjected to Analysis of Variance (Chi-square). Terminology of the stomata complex types used in this study is that of[19].

RESULTS AND DISCUSSION

Stomata complex types observed includes Anomocytic, (surrounded by a limited number of cells that are indistinguishable in size and shape from the other epidermal cells). In other types, the epidermal cells surrounding the stomata are differentiated as subsidiary cells. There were some with two subsidiary cells that are parallel to the guard cells (paracytic), or three subsidiary cells of unequal sizes (anisocytic), actinocytic (stomata surrounded by a ring of radiating cells), pericytic (where

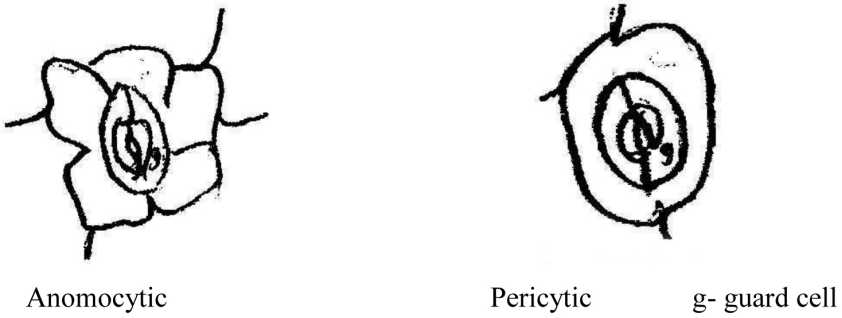

Fig. 1. Stomatal Complex Types in *Citrus limon*

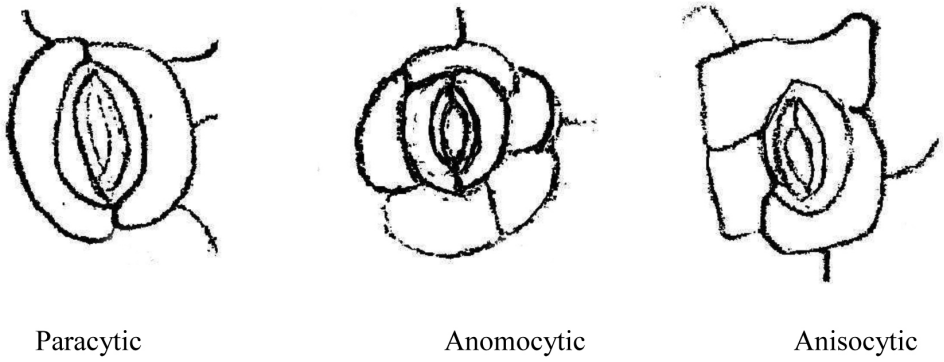

Fig. 2. Stomatal Complex Types in *Citrus sinensis*

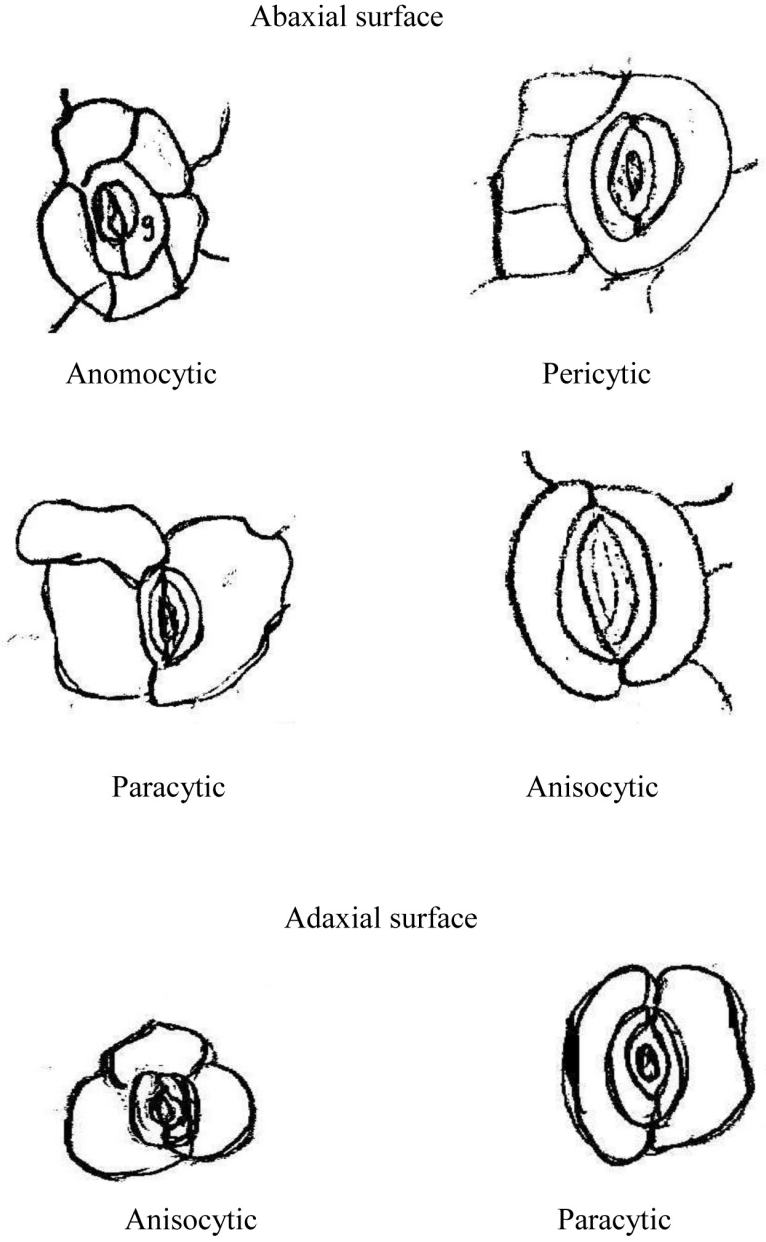

Fig. 3. Stomata complex types in *Eucalyptus torelliana*

a cell forms a ring around the stomata), cyclocytic (where a number of cells form a ring around the stomata) and tetracytic (where four specialized cells surround the stomata).

The stomatal complex types are heterogenous in all the four species studied. The heterogeneity varies from species to species. Two types of stomatal complex types were found in *C. limon (Fig. 1.),* three types in *Citrus sinensis* (Fig. 2), four types in *E. torrelliana (Fig. 3.)*, and five types in *E. camaldulensis* (Fig. 4.).

The leaves of the *Citrus* species studied were hypostomatic while those of Eucalyptus species were amphistomatic.

The range falls below the range established by [6] of 100-300 per mm^2 for leaves of many species. Higher stomatal density is usually associated with smaller stomatal [6]. The present study showed no conformity to this trend except in the Adaxial surfaces of *E. camaldulensis* and *E. torelliana*. Species with the highest stomatal densities also had the largest size. Stomatal density was highest in *C. limon ,* followed by *E. torelliana, C. sinensis,* and *E. camadulensis* [10], reported that some xerophytic plants have very high stomatal densities. Stomatal density generally showed positively correlation with transpirations. *C. limon* with the largest stomatal density (68.589) had the lowest transpiration rate ($1.296x10^{-5}$). *E. camaldulensis* with the density of 66.600 had transpiration rate of $3.606x10^{-3}$. Viewed in line with the advocacy of Citrus species for revegetation provenance efforts [5, 12], the Citrus species has proven superiority over the Eucalyptus species in having high stomatal densities with correspondingly large stomatal size, yet, having the lower transpiration rates. It is curious that *C. sinensis* with lower density than *C. limon* transpired more. This clearly shows the superiority of *C. limon* over *C. sinensis* in revegetation efforts.It is noted however, that

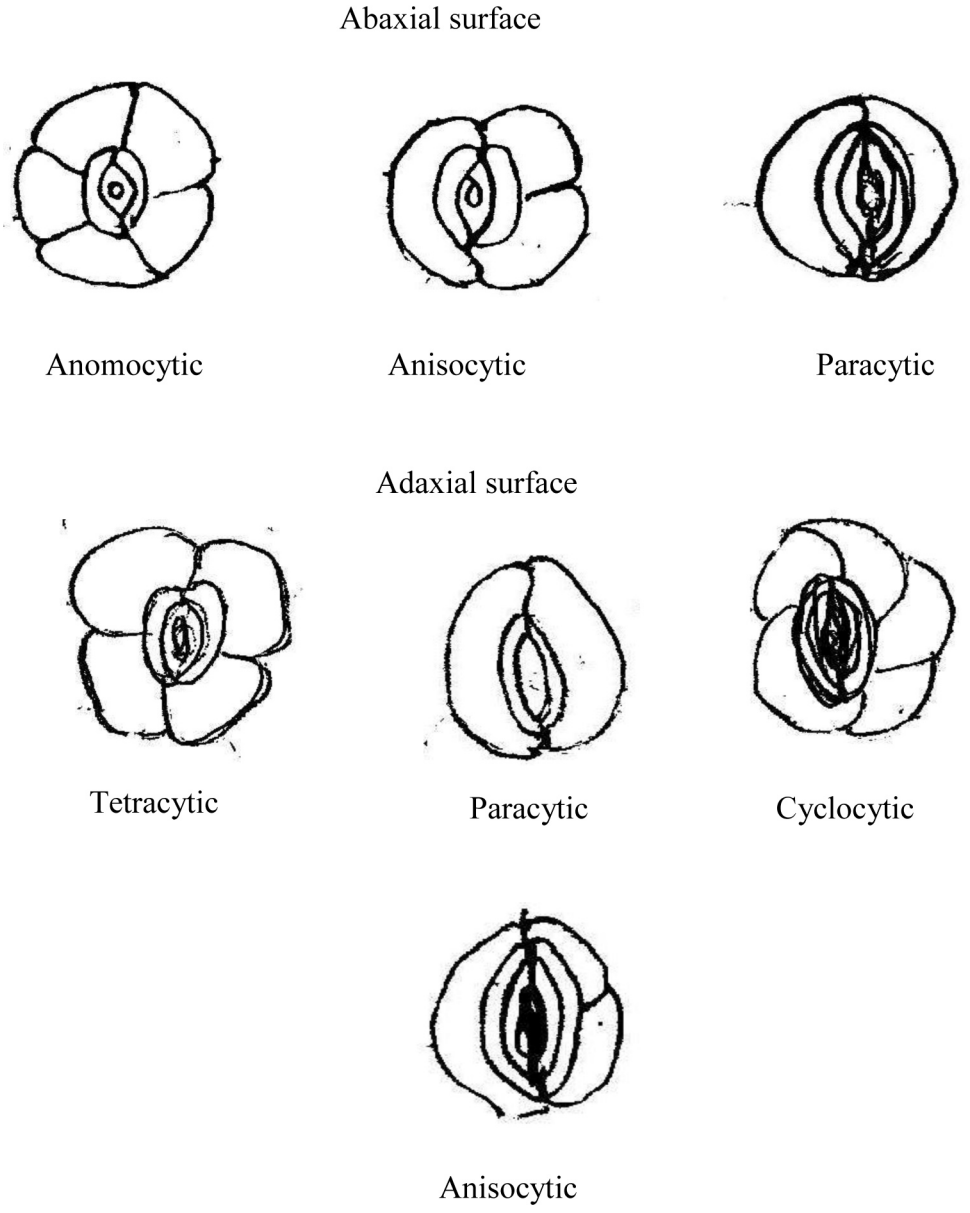

Fig. 4. Stomatal Complex Types in *Eucalyptus camaldulensis*

the citrus species are hypostomatic while the Eucalyptus species are amphistomatic. It is being suggested that the heterogeneity of the stomatal complex type may be one of the factors affecting stomata conductance. For example *E.torelliana* having the most heterogenous stomatal complex type (i.e. 5 types) transpired most, follow by *E.camaldulensis* having four types, *C. sinensis* (3 types)and *C. limon* having two transpired least. An earlier study by [20], showed that *Ficus sur*, *F.elastica*, and *F.vallis-choudae* with similarly heterogeneous stomatal complex types have high transpiration flux of water.

It is also interesting to note that *E.torelliana* and *E.camadulensis* having stomatal complex type with five or more epidermal cells having direct contact with guard cells, have transpiration rate that are higher than *C.sinensis* and *C.limon* having no such stomata. This view was corroborated in some citrus species by [12], some vegetable species by [21] and some afforestation trees species by [4]. This suggests that the level of transpiration rate may also be related to the number of epidermal cell having direct contact with the guard cells. [23] previously proposed that a large number of subsidiary cells may slow the stomatal opening process down.

The complex interplay of the stomatal features cum environmental factors has been discussed [3, 5, 7, 8, 22, 24]. The position of the stomata, whether hyperstomatic, hypostomatic or amphistomatic, have functional focus in the plant beyond what we measure as transpiration rate. Hydraulic conductance which employs massive transpiration may be the survival strategy of the plant to secure water absorption and conductance as well as prevent cavitation and air embolism. A systematic assessment of each species is therefore important in other to determine the usefulness or otherwise of transpiration rate in the plant. Also, environmental factors of temperature, humidity, water

Table 1. Stomatal parameters and transpiration rates in some members of Myrtaceae and Rutaceae

Species	Leaf surface	Types of stomata complex	Stomata size (µm)	Stomatal Density (mm^2)	Density of epidermal cells (µm)	Transpiration rate ($mol/m^{-2}/s^{-1}$)
Citrus limon	Abaxial	Anomocytic, pericytic	18.578a	68.589a	236.833a	1.296x10-5a
Citrus sinensis	Abaxial	Paracytic, anomocytic, anisocytic	19.021b	55.069b	266.767b	3.799x10-5b
Eucalyptus torelliana	Abaxial	Anomocytic, anisocytic, pericytic, paracytic	13.445a	66.600a	234.967a	3.606x10-3a
	Adaxial	Anomocytic, anisocytic, paracytic	19.035a	12.800a	281.067a	
Eucalyptus camadulensis	Abaxial	Anisocytic, paracytic, anomocytic	16.500b	51.967b	258.400b	3.152x10-3b
	Adaxial	Tetracytic, paracytic, cyclocytic, anisocytic	13.397b	27.067b	279.633b	

availability in the soil must be adjudged with transpiration rate (and also the hydraulic conduit) in order to assess each species' survival ability.

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