

Leaf Epidermal Characteristics of Four Species of the Genus *Citrus* (Rutaceae)

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Abstract: Studies on the leaf epidermal characteristics of 4 species of *Citrus* namely: *C. sinensis*, *C. limon*, *C. aurantifolia* and *C. paradisi* were carried out to investigate and compare the leaf epidermal features and possibly establish the taxonomic relationships among these taxa. The results showed that at the lower leaf epidermis, the epidermal cells of *C. sinensis* and *C. aurantifolia* are irregular but vary from pentagonal to hexagonal in *C. limon* and rectangular to pentagonal in *C. paradisi*. Also, at the upper leaf epidermis, the epidermal cells of *C. sinensis* and *C. paradisi* are irregular, pentagonal in *C. aurantifolia* but vary from rectangular to pentagonal in *C. limon*. Both taxa showed interspecific similarities in anomocytic stomata, absence of stomata at the upper leaf epidermis, absence of trichomes, absence of subsidiary cells and thick walled cells that characterized all the species. The similarities in structures are strong reasons for the 4 taxa to be in the same genus while the differences suggest reasons for the existence of each as a separate species and thus strengthen the reliability of leaf epidermal features as taxonomic tools.

Key words: Leaf, epidermal characters, species, *Citrus*, rutaceae

INTRODUCTION

The genus *Citrus* is an evergreen tree belonging to the family Rutaceae. They are mostly shrubs with very hard wood and often have spines. It has about 150 genera and 1500 species (Cobley, 1976). *Citrus* fruit is a special kind of berry (Hesperidium) filled with stalked spindle-shaped pulp vesicles (juice sacs) covered by a white spongy tissue (albedo) and a peel with numerous oil glands, turning yellow or orange at full maturity (Okwulehie, 1998).

Citrus have sweet smelling flowers and are borne in small groups in the leaf axil of young twigs. The petals are white and often variable in number. The sepals are small, greenish and 5 in number. The stamens are more or less numerous and are in 5 whorls. The filaments of each whorl are partially united at the base. The ovary is one consisting of 8-15 united carpels each containing several ovules in axil placentation. The style is short and deciduous. The pollen grains are sticky and flowers are self and cross pollinating (Davies, 1994).

Citrus fruits are among the most important fruit crops in the subtropical regions. They are used in making fresh juice and flavouring. The oil "citral" and "limonene" extracted from lemon and sweet orange, respectively are used in making perfumes (Reuther, 1976). Extracts from lime is used as cleansing agent. The waste pulp is used for feeding cattle, producing pectin and vitamin C.

Medicinally, the leaves of sweet orange are used in treating swollen limbs, the juice from lemon is used for treating hookworm and the juice from lime is used in treating dermatitis (Okeke and Mbagwu, 2001).

In recent time, leaf epidermal features have received very considerable attention by taxonomists (Stace, 1980; Ayensu, 1970b). Following this discovery, epidermal features became widely studied from three main perspectives: ontogenetic, phylogenetic and taxonomic. Ontogenetic investigations have tended to show methods of origin of the different stomatal 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 (Vancotthem, 1970; Pant, 1965).

The use of epidermal characters such as stomatal 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 cytology and genetics cannot be applied (Stace, 1965a). In dicotyledons, the systematic value of epidermal and cuticular features have been indicated. For example, Stace (1965a) made extensive comparative

studies on tropical mangrove families, Combretaceae, Rhizophoraceae and Avicenniaceae. Also, at the genus level, the presence of subsidiary cells is very important, hence the genus *Str ephonema* with two subsidiary cells surrounding the guard cells can be easily distinguished from other genera without subsidiary cells (Stebbins and Khush, 1961). The most recent method is the study of leaf surfaces by means of scanning Electron Miroscope (Cutler, 1978). This technique is particularly useful because it reveals details of the topography of both the outer and the inner layers of the leaf.

Many workers showed that leaves possess many morphological attributes of potential taxonomic significance that are often diagnostic at the genus and specie levels (Edeoga, 1991; Arroyo, 1985; Mbagwu and Edeoga, 2006). The shape of epidermal cells, types and arrangement of stomata and size and shape of trichomes are important systematic parameters. A general review of the tribal, generic and specific characters was made in the family Combretaceae by Stace (1965a) based on the characteristics of the leaf epidermal morphology alone. Mbagwu (2005) studied the leaf epidermal features of *Vigna* sp. and separated *V. gracillis* and *V. racemosa* from other species based on trichome morphology. Other workers have used leaf epidermal characters to elucidate the problem of recognition and identification of some members of the family Costaceae, Onograceae, Leguminosae-papilionoideae and Metastomataceae (Edeoga and Eboka, 2000; Mbagwu and Edeoga, 2006).

This study therefore, reports the leaf epidermal characteristics of four species of the genus *Citrus*. It also, assesses the systematic importance of and discusses the extent to which, leaf epidermal features may be utilized in the biosystematic consideration of these species in view of their perceived similarities in structural and reproductive biology.

MATERIALS AND METHODS

Fresh leaves of *C. sinensis*, *C. limon*, *C. aurantifolia* and *C. paradisi* were collected from different plant gardens in Owerri, Imo State, Nigeria. They were confirmed and identified at Imo State University Herbarium Owerri, Nigeria. The leaves were washed in tap water. This study was carried out in March, 2007 at Plant Science and Biotechnology Laboratory, Imo State University, Owerri, Nigeria. Each leaf were painted with finger nail polish on both the upper and lower surfaces and allowed to dry. After drying, a short clear cellophane tape were firmly pressed over the dried nail polish on the surfaces. The tape were carefully peeled from the leaf and affixed on a clean slide and observed under the Leitz

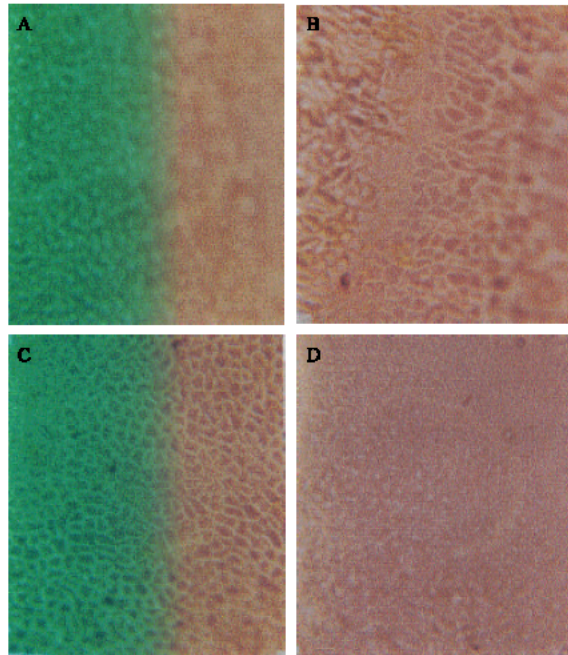


Fig. 1 (a-d): Upper leaf epidermis of the four *Citrus* species studied, a- *C. sinensis*, b- *C. limon*, c- *C. aurantifolia*, d- *C. paradisi*

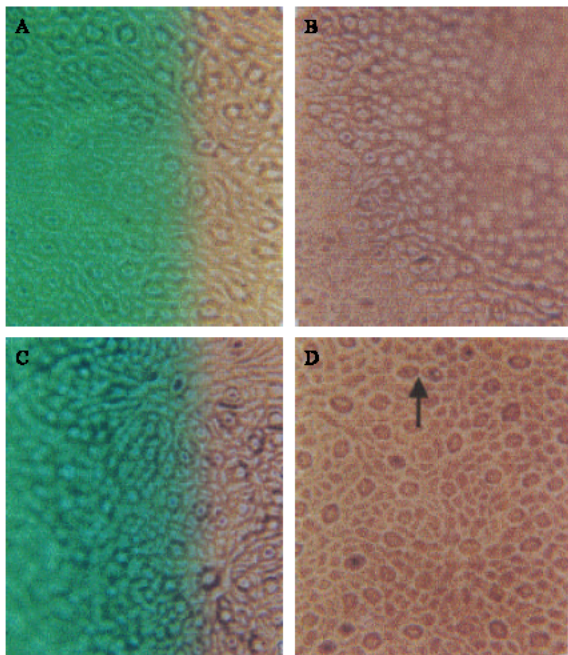


Fig. 2 (a-d): Lower leaf epidermis of the four *Citrus* species studied, a- *C. sinensis*, b- *C. limon*, c- *C. aurantifolia*, d- *C. paradisi*

Wetzler Ortholux Microscope fitted with vivitar-V-335 camera. The type of stomata and shape of epidermal cells

Table 1: Upper leaf epidermal characteristics of *Citrus* species studied

Characters	<i>C. sinensis</i>	<i>C. limon</i>	<i>C. aurantifolia</i>	<i>C. paradisi</i>
Stomatal type	None	None	None	None
Number of stomata	None	None	None	None
Number of subsidiary cell	None	None	None	None
Shape of epidermal cell	Irregular	Rectangular to pentagonal	Pentagonal	Irregular
Number of epidermal cell	315-345	310-338	315-330	310-337
Trichomes	Absent	Absent	Absent	Absent

Table 2: Lower leaf epidermal characteristics of *Citrus* species studied

Characters	<i>C. sinensis</i>	<i>C. limon</i>	<i>C. aurantifolia</i>	<i>C. paradisi</i>
Stomatal type	Anomocytic	Anomocytic	Anomocytic	Anomocytic and contiguous stomata
Number of stomata	203-211	200-208	201-207	200-207
Number of subsidiary cell	None	None	None	None
Shape of epidermal cell	Irregular	Pentagonal to hexagonal	Irregular	Rectangular to pentagonal
Number of epidermal cell	300-310	285-308	301-307	295-305
Thickness of cell	Thick walled	Thick walled	Thick walled	Thick walled
Trichomes	Absent	Absent	Absent	Absent

were determined according to Vancotthem (1970). Also, the number of stomata, epidermal cells and other epidermal features were observed (Table 1 and 2). Photomicrographs illustrating the epidermal features were taken from the slides using Leitz Wetzler Ortholux microscope fitted with vivitar-V-335 camera (Fig. 1 and 2).

RESULTS

The results of this investigation showed some similarities and differences that are taxonomically important. For example, both taxa lacked stomata, subsidiary cells and trichomes at the upper leaf epidermis but approximately with the same number of epidermal cells (Table 1). The shape of epidermal cells is irregular in *C. sinensis* and *C. paradisi* but vary from rectangular to pentagonal in *C. limon* and pentagonal in *C. aurantifolia* (Table 1).

The results at the lower leaf epidermis also showed an interesting similarities and differences. For example, both taxa possessed anomocytic type of stomata, approximately the same number of stomata and epidermal cells in each taxon, absence of subsidiary cells and trichomes and with characteristic thick walled cells (Table 2). The shape of the epidermal cell is irregular in *C. sinensis* and *C. aurantifolia*, vary from pentagonal to hexagonal in *C. limon* and rectangular to pentagonal in *C. paradisi* (Table 2).

DISCUSSION

The results of this investigation showed that leaf epidermal features are useful in characterization and determination of interspecific relationships among the four taxa investigated. The variation in the shape of the epidermal cells is not strange since Olowokudejo (1990) made similar observation in some *Annona* sp. pointing out

that *A. squamusa* is characterized by sinuate epidermal cells whereas other investigated *Annona* species have irregular epidermal cells with curved antidual walls. This observation is similar among the *Citrus* species investigated since *C. sinensis* and *C. aurantifolia* have irregular epidermal cells at the lower leaf epidermis suggesting close affinity between them.

Also the lack of stomata in both taxa at the upper leaf epidermis and the hypostomatic nature of both taxa at the lower epidermis (Table 1 and 2) are probably an adaptation to water loss and ecological advantage to enable these plants to survive as perennial plants. This is in line with the work of Gold-Schmidt (1996) and Shaw (1977) who carried out investigations concerning the loss of water from *Citrus* leaves and some of the factors governing it. An important aspect of this work is that it is in line with the result of other workers that the family Rutaceae is characterized by anomocytic stomata (Dugo, 2002; Stebbins and Khush, 1961). The leaf epidermal characteristics of these taxa have shown that this line of evidence is taxonomically important, suggesting that similarities in structures showed interspecific relationships while differences in structures showed no relationship among the investigated taxa.

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