

COMPARATIVE ANATOMICAL STUDIES OF THE STOMATAL PATTERNS OF SOME TREE SPECIES OF STERCULIACEAE AND VERBENACEAE IN NIGERIA

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Abstract

Leaf epidermal preparations of seven species within the Sterculiaceae and Verbenaceae were examined with light microscope to determine stomata patterns and other features of taxonomic importance. Impression technique was conducted on freshly harvested leaves to reveal important foliar epidermal characters. The stomata size, index and cell wall thickness were measured following standard procedures. Stomata were only seen in the abaxial surface of all the species, with four types observed; anomocytic, paracytic, diacytic and anisocytic. Anomocytic type was recorded for *Duranta erecta*, *Tectonia grandis* and *Vitex doniana* while *Gmelina arborea* had diacytic type. Sterculiaceae species showed anomocytic in *Cola gigantea*, anisocytic in *Theobroma cacao* and paracytic in *Cola rostrata*. Stomata sizes, numbers and epidermal cells varied across the families. Stomatal size, index and wall thickness varied greatly within the species. Variations and similarities observed in the stomatal features provide evidence of the genetic and evolutionary relationships and therefore are of taxonomic importance.

Key words: Anatomical studies, Stomatal patterns, Tree species, Sterculiaceae, Verbenaceae.

Introduction

Plants perform various physiological processes during the days and in the nights with some of their vital organs. Arguably the most important of these physiological activities are photosynthesis, transpiration and gaseous exchange, and they all take place in the leaf of the plant (Fowden *et al.*, 1993). Stomata are minute apertures formed particularly in the epidermal layer of the leaves and other green aerial parts of the plant. The stomatal complex comprises of the stoma (the stomatal opening), surrounded by two semi-lunar cells, known as the guard cells, which guard and regulate the opening and closing of the stoma. Sometimes the guard cells are surrounded by two or more cells which are distinct from the other epidermal cells; such cells are called accessory cells or subsidiary cells (Esau, 2006). In dicotyledonous leaves, the stomata remain scattered, while in monocotyledonous leaves, they occur in parallel rows. The number of stomata on leaf surfaces varies widely among different species of plants, but generally, the lower epidermis of the leaf tends to have more number of stomata than the upper surface (Croxdale, 2000).

Apart from their roles in transpiration and gaseous exchange, plant stomatal patterns and distribution have been found very useful as diagnostic tools in plant taxonomy and systematic (Mashile & Tshisikhawe, 2017). Together with other leaf micro-characters stomatal parameters such as type, size, cell wall thickness, index and occurrence have been used for taxonomic distinction and recognition in the angiosperm families (Sonibare & Adeniran, 2014), and for distinguishing crude drugs even when they are fragmented or powdered. Diversity in stomata was first studied by Stresburger and then by Vesque who recognized four broad categories based on the presence and arrangement of subsidiary cells as well as their

mode of development. These stomata are broadly classified into four (4) basic types namely; anomocytic (ordinary epidermal cells surrounding the stomata – subsidiary cell absent), anisocytic (three subsidiary cells of unequal size surrounding the stomata), paracytic (two subsidiary cells arranged parallel to guard cells) and diacytic (two subsidiary cells at right angles to the guard cells). Other categories, even though less common in nature include antinocytic type (stomata surrounded by a ring of radiating cells) and cyclocytic type (with four subsidiary cells) (Esau, 2006; Pandey & Misra, 2008). More than one type may sometimes occur together on the same organ (Kagan & Sachs, 1991; Croxdale *et al.*, 1992). Stomata may occur on both sides of the leaf (amphistomatous leaf) or on either surface alone, more often on the lower surface (hypostomatous leaf). Similarly, although rare, some floating plants bear stomata only on the upper surface (epistomatous) of the leaves (Camargo and Marenco, 2011; Mashile & Tshisikhawe, 2017).

Verbenaceae (Verbena family) is a large family of plants distributed across a diverse habitat. They commonly occur as lianas, but trees, herbs and shrub have also been recorded (Atkins, 2004; Marx *et al.*, 2010). In Nigeria, the family is represented by ten (10) native genera (*Stachytapheta*, *Verbena*, *Vitex*, *Lantana*, *Premna*, *Clerodendrum*, *Lippia*, *Phyla* and *Priva*) and four (4) introduced ornamentals (*Gmelina arborea* Roxb., *Tectona grandis* L.f., *Duranta repens* L. *Holmskioldia sanguinea* Retz. and *Petrea volubilis* L.) (Hutchinson & Dalziel, 1963). They are cultivated in gardens as important ornamentals and also used in herbals (Meena *et al.*, 2011). *Tectona grandis* and *Gmelina arborea* are popular woods in timber industry while *Vitex doniana* leaves are valued as vegetable in Nigeria and the wood used as fuel in cooking and bakery (Burkill, 1985).

Sterculiaceae is another large family of interest. They occur as trees, shrubs or herbs, and are predominantly sub-tropical to tropical. Leaves are simple, having opposite or whorled arrangement and mostly entire and stipulate. The leaf shapes varied from elliptic, lanceolate to palmate but rarely obviate (Hutchinson & Dalziel, 1963). The leaves are mostly glabrous in all except *Cola hispida*, *Malachanta alnifolia*, *Mansonia altissima* and *Waltheria indica*. Members of the Sterculiaceae are commercially exploited for their seed output. *Theobroma cacao* is used in industrial production of chocolate and its products. *Cola accuminata* and *Cola hispida* seeds are highly priced for their caffeine contents. So many other members have been heavily utilized in traditional remedies for various ailments, as firewood and timber for construction (Camargo & Marenco, 2011).

It is evident that the members of these plant families, especially the tree species are of high economic importance and therefore need complete taxonomic evaluation for their proper utilization and conservation. It was therefore on this note that this present study was initiated. The stomatal characters were studied in three species of Sterculiaceae (*Cola gigantea*, *Cola rostrata* and *Theobroma cacao*) and four species of Verbenaceae (*Duranta erecta*, *Gmelina arborea*, *Vitex doniana* and *Tectonia grandis*).

Materials and Methods

Four genera from Verbenaceae and three genera from Sterculiaceae were selected for the study. Fresh samples used for these investigations were collected from University of Nigeria Botanic Garden (*Duranta erecta*, *Tectona grandis*, *Cola gigantea* and *Cola rostrata*), University of Nigeria Medical Centre (*Vitex doniana* and *Gmelina arborea*) and at Enugu Ezike (*Theobroma cacao*), all in Nsukka area of Enugu State, Nigeria. They were all properly identified and voucher specimens deposited in the herbarium of the Department of Plant Science and Biotechnology, in the University of Nigeria, Nsukka.

Impression technique (Hilu & Randall, 1984) was adopted in this study. This was carried out to find the type of stomata present; this was done with nail varnish, forceps, slides, cover slips, light microscope and Moticam attachment. The fresh leaves were plucked, washed and cleaned, nail varnish was applied to give a thicker surface on both the adaxial and abaxial surfaces and was left to dry for one hour. After drying, the impression left on the polish film produce an excellent detailed image of the epidermis and the surfaces were brought out with the aid of forceps and then placed on microscope slides. The casts were mounted for examination by light microscopy and were prepared for photomicrography as described by Aworinde & Ogundairo (2009). Photomicrographs of the specimens were taken in the Faculty of Veterinary medicine, University of Nigeria, Nsukka. Stomata and epidermal counts were aided by the moticam and measurement of parameters for morphological data followed by Isawunmi (1989) and observation of quantitative characters was made *in situ*.

Stomata index (SI) was calculated using the formula of Salisbury (1972) as modified by Hussin *et al.*, (2000).

$$\text{Stomatal index} = S / (S+E) \times 100$$

where S denotes number of stomata in each unit area and E number of epidermal cells in the same unit area. To measure the width of the epidermal cell, the widest points of each cell was taken.

The dimensions were measured using a light microscope. The ocular tube of the microscope was fitted with an ocular micrometer which was calibrated using a 2 mm range stage micrometer. This was done by aligning the zero marks of the stage micrometer with that of the ocular. The number of units of the ocular, which aligns with a given unit of the stage micrometer at 400x magnification, was noted. This was used as the conversion factor in the subsequent measurements.

At 400x magnification

44 units of the ocular = 0.15

1 unit of the ocular = 0.15 = 0.003mm/44

Therefore, conversion factor at 400x magnification = 0.003mm

The dimensions measured were: Stomata length, Stomata width, Stomata cell wall thickness.

The data collected from the stomatal measurements of the different species and the variations among the stomatal dimensions of the abaxial surfaces of species of the two families (Verbenaceae and Sterculiaceae) were analysed using one way analysis of variance.

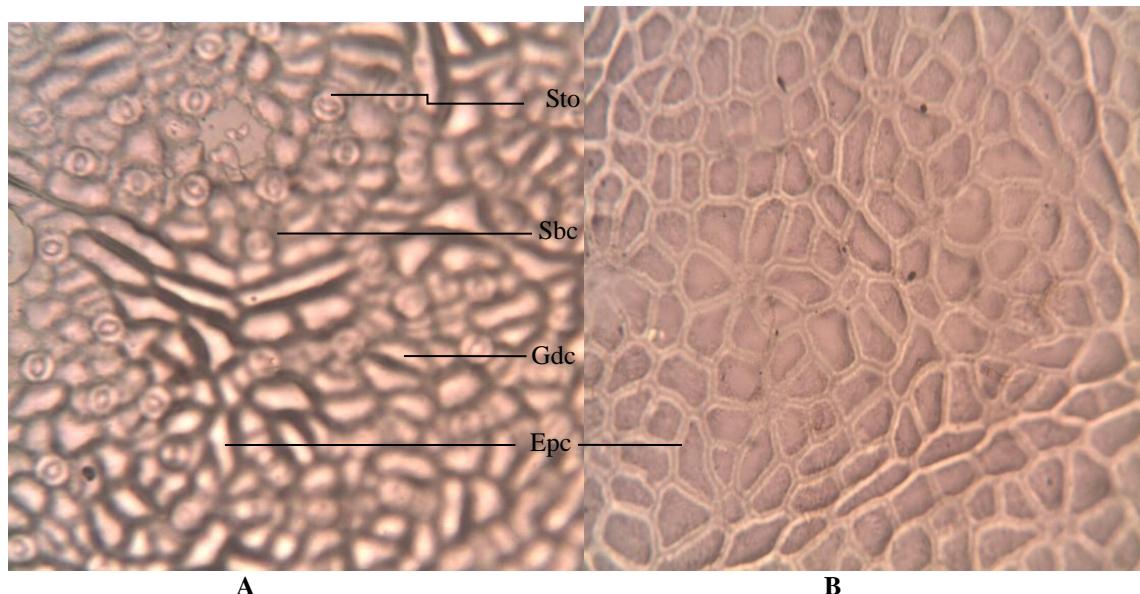
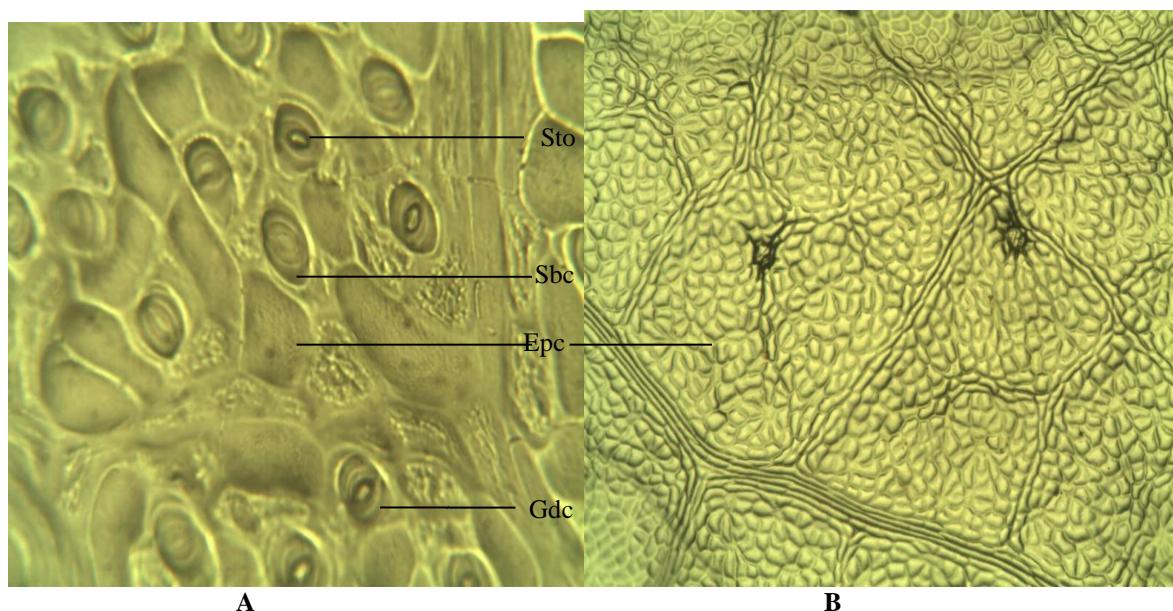
Results

Parameters of the stomatal characters are presented in Table 1 while Plates (1-14) show the images from the photo micrographic study.

The most common type of stomata in Sterculiaceae family was paracytic as found in *Cola rostrata*; anisocytic and anomocytic were found in *T. cacao* and *C. gigantea* respectively. In Verbenaceae, the most common stomata type was diacytic as found in *Gmelina arborea* and occasionally anomocytic as found in *D. erecta*, *V. doniana* and *T. grandis*, the stomata were only on the abaxial surfaces (Plates 1-14) in the two families. There were significant differences in both the mean stomata length and mean stomata cell wall thickness between *Cola rostrata* and *Theobroma cacao* and between *Theobroma cacao* and *Cola gigantea* but there were no significant differences between *Cola gigantea* and *Cola rostrata* and for the mean stomata width, there were significant differences among the three species of Sterculiaceae. For Verbenaceae, there were significant differences among the species in the three stomata dimensions, exceptions included; the mean stomata length where there was no significant difference between *Tectona grandis* and *Duranta erecta*, the mean stomata width and cell wall thickness where significant differences between *Gmelina arborea* and *Duranta erecta* were observed.

Table 1. Stomata character of the studied taxa [mm (min. (mean ± S.E.) max] abaxial surface.

Taxa	Stomata length per mm	Stomata width per mm	Cell wall thickness per mm	Stomatal index %
<i>Cola rostrata</i>	0.012 (0.023 ± 0.0011) 0.03	0.002(0.003 ± 0.0003) 0.005	0.002 (0.003 ± 0.0003) 0.005	22.31
<i>Theobroma cacao</i>	0.009 (0.011 ± 0.0004) 0.01	0.000(0.001 ± 0.0001) 0.002	0.000 (0.001 ± 0.0001) 0.002	21.37
<i>Vitex doniana</i>	0.015 (0.018 ± 0.0007) 0.024	0.001(0.003 ± 0.0004) 0.006	0.001 (0.003 ± 0.0004) 0.006	15.85
<i>Cola gigantean</i>	0.018 (0.021 ± 0.0012) 0.033	0.001(0.003 ± 0.0005) 0.008	0.001 (0.003 ± 0.0005) 0.008	18.22
<i>Gmelina arborea</i>	0.005 (0.007 ± 0.0003) 0.009	0.000(0.000 ± 0.0000) 0.000	0.000 (0.000 ± 0.0000) 0.000	20.61
<i>Duranta erecta</i>	0.099 (0.013 ± 0.0005) 0.017	0.001(0.001 ± 0.0000) 0.001	0.001 (0.001 ± 0.0000) 0.001	18.56
<i>Tectona grandis</i>	0.009 (0.014 ± 0.0007) 0.019	0.009 (0.013 ± 0.0004) 0.017	0.009 (0.013 ± 0.0004) 0.017	20.62

Plate 1. (A) Abaxial surface and (B) Adaxial surface of *Theobroma cacao* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.Plate 2. (A) Abaxial surface and (B) Adaxial surface of *Cola gigantea* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.

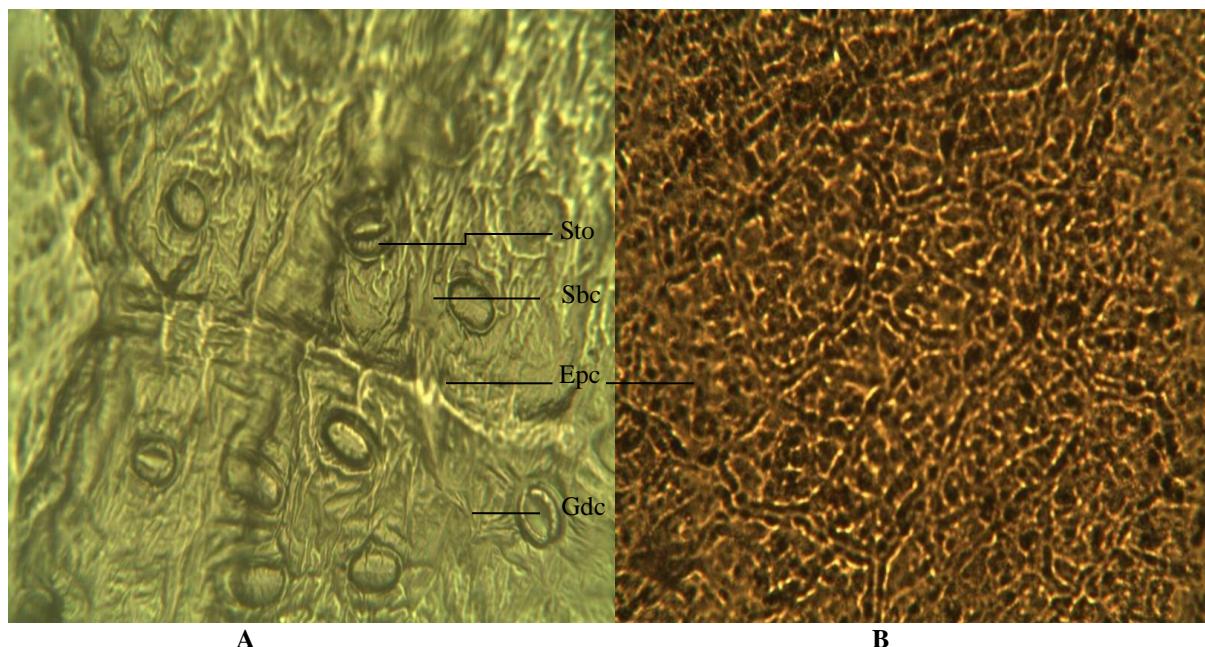


Plate 3. (A) Abaxial surface and (B) Adaxial surface of *Cola rostrata* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.

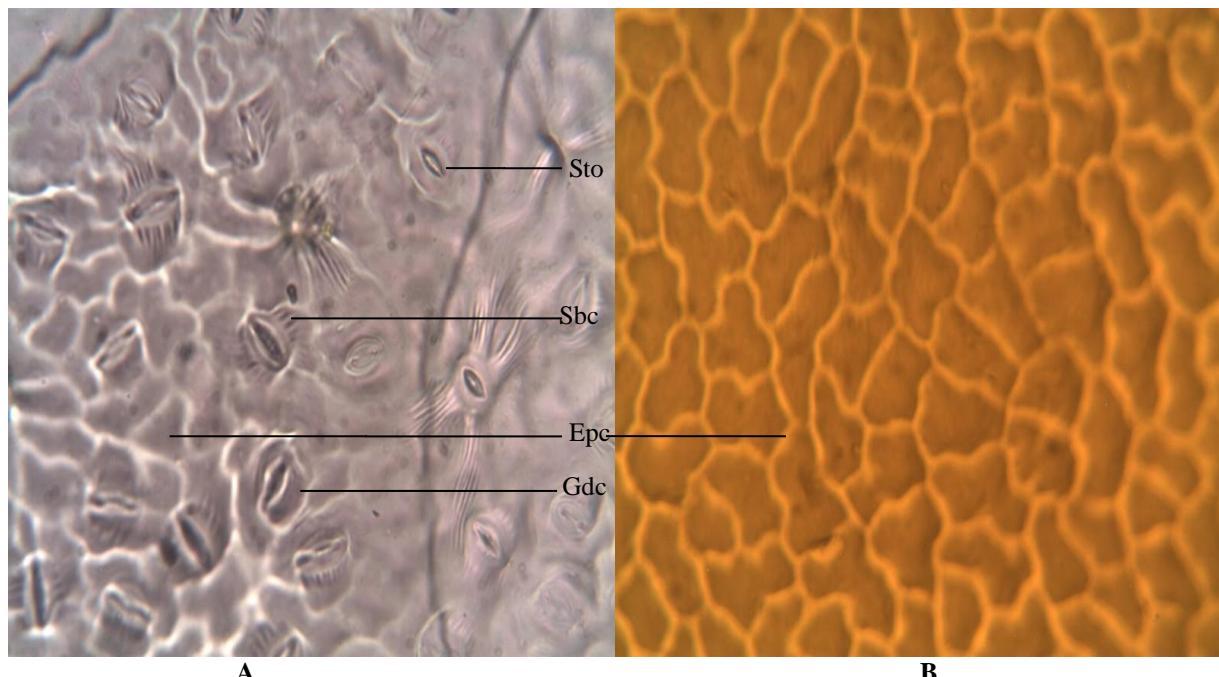


Plate 4. (A) Abaxial surface and (B) Adaxial surface of *Duranta erecta* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.

Discussion and Conclusion

The study of the stomatal pattern of some species of Sterculiaceae and Verbenaceae revealed a number of important stomatal characters and these characters exhibited interesting inter-specific variations that were of diagnostic significance for identification and delimitation of species within the taxa. There was a wide variation in the number and distribution of stomata found in all the species studied. Classification of different types of stomata complexes was based on the number and position of the subsidiary cells. There was an occurrence of

anomocytic type in almost all the species of Verbenaceae studied, except in *Gmelina arborea* where diacytic stomatal type was encountered, whereas there was presence of anisocytic and paracytic stomata in *T. cacao* and *C. gigantea* respectively. This has in agreement with Aworinde *et al.*, (2012) who reported foliar epidermal characters in some species of Sterculiaceae in Nigeria.

All the plants studied showed hypostomatic leaves. The appearance of more stomata on the abaxial (lower) surface could be an adaptation to water loss as suggested by Mbagwu *et al.*, (2008) and Aworinde & Ogundairo (2009) who stressed that hypostomatic leaves are adaptive

feature to survive drought. This result further agrees with the earlier findings of Metcalfe & Chalk (1950) and Mbagwu & Edeoga (2006).

The differences reported in the quantitative measurement of other parameters (stomatal size, cell wall thickness and stomata index) could be attributed to a reflection of physiological responses to a number of environmental factors and these differences could be usefully adopted as diagnostic characters in their

delimitation at the species level (Adegbite, 2008; Aworinde *et al.*, 2012).

The variations observed in the data and figures of the characters such as stomata sizes and stomata index of the species studied are therefore of taxonomic importance. Also the similarities in the observed species provided evidence for their genetic and evolutionary relationships and to an extent, justification for their taxonomic grouping.

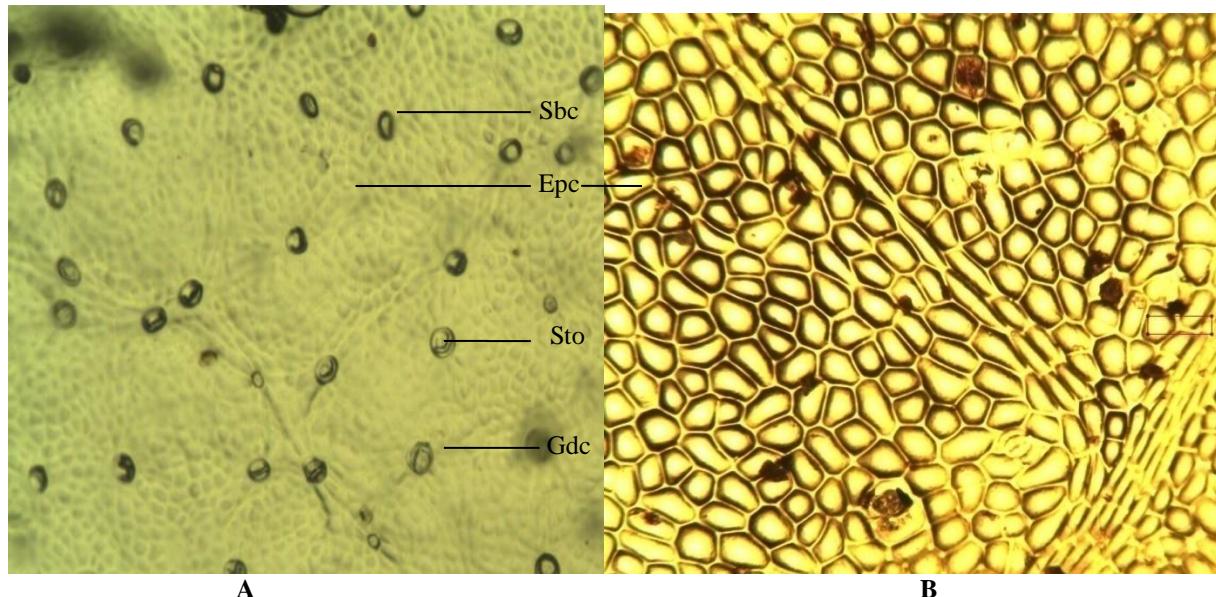


Plate 5. (A) Abaxial surface and (B) Adaxial surface of *Gmelina arborea* (X40)
Sto = Stoma; Gdc = Guard cell; Sbc = Subsidiary cell; Epc = Epidermal cell.

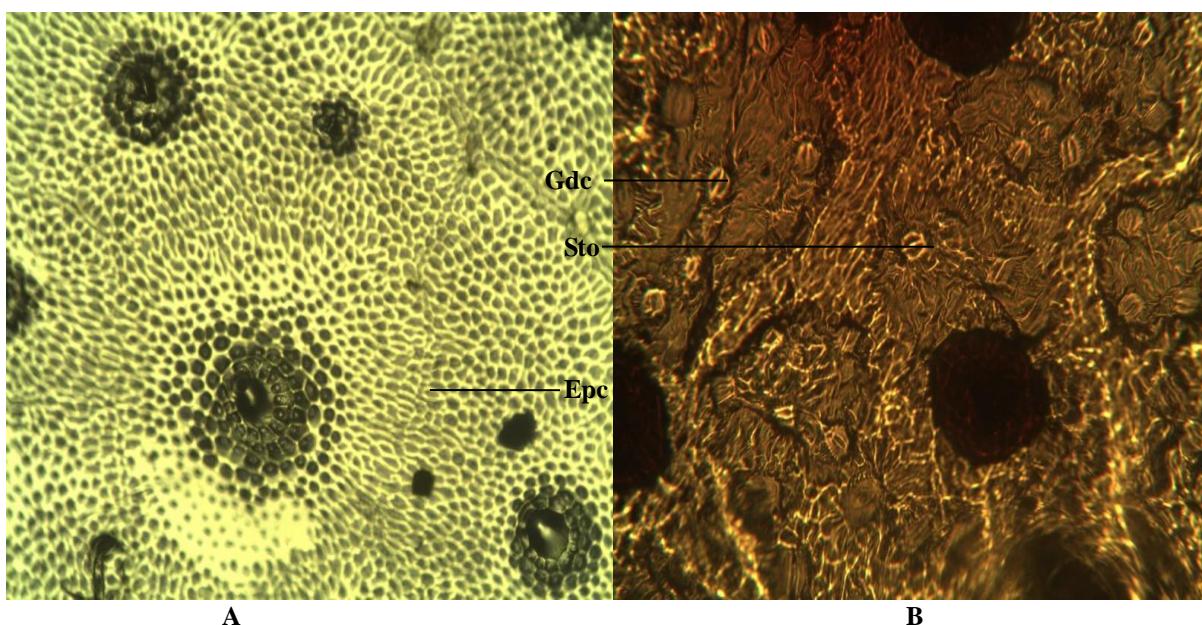


Plate 6. (A) Adaxial surface and (B) Abaxial surface of *Tectona grandis* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.

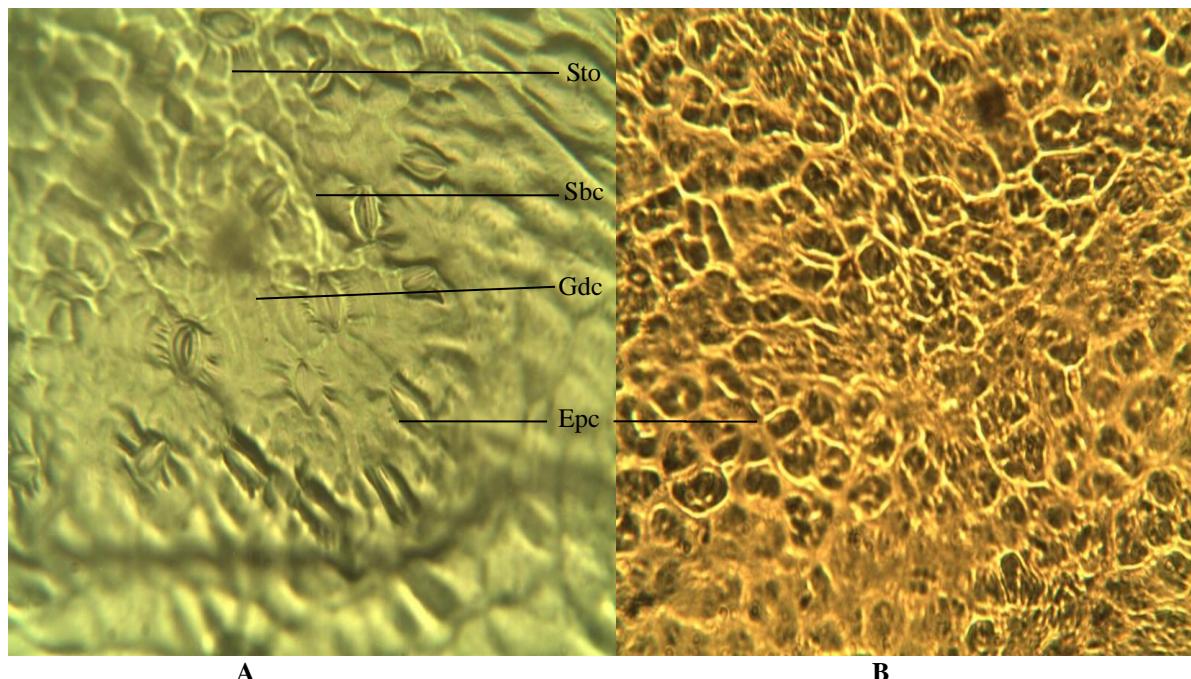


Plate 7. (A) Abaxial surface and (B) Adaxial surface of *Vitex doniana* (X40)
Sto = Stoma; Sbc = Subsidiary cell; Gdc = Guard cell; Epc = Epidermal cell.

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(Received for publication 27 March 2017)