J. Plant Develop.
19(2012): 13-22

LEAF ANATOMICAL VARIATION IN RELATION TO STRESS TOLERANCE AMONG SOME WOODY SPECIES ON THE ACCRA PLAINS OF GHANA

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Abstract: Leaf anatomical study was conducted on some woody species on the Accra Plains of Ghana. Leaf epidermal strips and transverse sections were mounted in Canada balsam and studied. The anatomical studies revealed numerous stomata on the lower epidermis of *Azadirachta indica*. The anatomical studies revealed the presence of thick cuticles, double-layered palisade mesophyll in most species and the presence of epidermal hairs in some species. *Ficus capensis* showed the presence of cystolith in the lower epidermis whereas *Zanthoxylum zanthoxyloides* showed the presence of mucilage gland in the upper epidermis. Epidermal cell of *Chromolaena odorata* are very large with undulating cell walls. The species studied had various adaptive anatomical features. The stomatal frequency of *Azadirachta indica* was very high. With the exception of *Chromolaena odorata* the stomatal frequencies of the species were relatively high. The stomatal dimensions showed that most of the species maintained constant stomatal length during the study period except *Griffonia simplicifolia* that increased the stomatal width during the afternoon. Unlike *Morinda lucida*, *Griffonia simplicifolia* and *Chromolaena odorata*, that showed reduction in the breadth of stomata, the other species maintained constant stomatal width.

Key words: leaf anatomy, stomatal dimension, woody species, Accra Plains, drought stress

Introduction

The morphological features (both external and internal) and physiological responses are linked to adaptive characteristics of plants in stressed environments. There is a close relationship between the thickness of leaves and degree of cutinization of their outer epidermal wall on one hand and the extent to which the underlying tissues are required to be protected against excessive transpiration on the other hand [HABERLANDT, 1928]. In some plant species, the upper epidermis is often thicker-walled than the lower epidermis [YANNEY-WILSON, 1963]. It is obvious that the upper surface receives more light and heat hence requires more effective protection against evaporation [EHLERINGER & MOONEY, 1978]. The outer walls of epidermal cells may be coated with wax so as to avoid the capillary occlusion of stomata and in some species; each stoma is surrounded by a ring of wax which thus forms an external air-chamber [MULROY, 1979].

The presence of leaf pubescence has long been positively associated with arid climate conditions. It has been shown to increase light reflectance from the leaf surface [EHLERINGER & MOONEY, 1978]. The boundary layer also affects leaf temperature by modifying the rate of heat transfer from the leaf [EHLERINGER & MOONEY, 1978]. The hairy cover is also reported to impede surface-ventilation by producing a labyrinth of spaces filled with stationary air [HABERLANDT, 1928].

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Many plants such as *Nauclea latifolia*, *Zanthoxylum zanthoxyloides*, *Ficus capensis* and *Azadirachta indica* are also reported to develop smooth and shinning upper leaf surfaces. Such polished surfaces protect the leaf against excessive insolation, which undoubtedly may prevent certain proportions of the incident light from penetrating into the leaf. However, smooth epidermal surfaces are often susceptible to wetting; but the deposition of wax protects the surfaces from wetting. Also the external resin coating of leaves conserve water by reducing non-stomatal transpiration [BAZZAZ & al. 1987].

The presence of thick cuticle and dense cell structure are inherently the modifications possessed by plant species for water conservation and also to withstand temperature stress [TRESHOW, 1970; MOONEY & GULMON, 1979]. The presence of hypodermis has been indicated as evolutionary solution to water stress [MOONEY & GULMON, 1979]. TRESHOW (1970) indicated that xerophytic conditions often reduce cells size, and intercellular spaces in leaves are less extensive. The increase in palisade mesophyll layer and reduction in the spongy mesophyll layer are modification to withstand drought stress. Drought stress has been indicated to increase the mechanical tissues for plants so as to be able to withstand the mechanical stress of desiccation.

The presence of mucilage in some cells and also in the cell wall of some species serves to conserve much water during water stress. The mucilage swells in water to form loose gels or slimy mass [GREEN & al. 1986]. The aim of this study is to determine the leaf anatomical variation in relation to stress tolerance among some woody species on the Accra Plains.

Study site. The Accra Plains is a triangular area in the southeastern part of Ghana. It covers an area of about 2,800 km^2 [JENIK & HALL, 1976] being bordered on the east by the lower reaches of the Volta river, the west by Winneba, the north by the Akwapim Scarp, and the south by the Gulf of Guinea. The plains is not a homogenous area, thus JENIK & HALL (1976) have divided it into seven (7) smaller geographical units based on the vegetation and soil types. The northern boundary is a hilly forest whereas the eastern and western boundaries are the Guinea savanna. Although the vegetation of the Accra plains is referred to as savanna, it does not fit into any of the main savanna types found in West Africa; hence might better be referred to as a kind of steppe since the grasses in this very dry area of less than 750mm rainfall rarely exceed 80 cm in height [LAWSON & JENIK, 1967; JENIK & HALL, 1976].

The plains is spatially isolated from the other savanna areas and its anomalous dry climate with a combination of low rainfall, moderate and rather high humidity has been designated the “Accra-Togo Dry Coastal Climate”, rendering it of special ecological interest [BRAMMER & DE ENDREDY, 1962; HARRISON CHURCH, 1963; JENIK & HALL, 1976].

The Accra Plains is obviously under stress due to (i) the low rainfall [BENNEH & AGYEPONG, 1990] and (ii) the increase in population on the plains (in general) and also in the vicinity of the study site, Pinkwae. This study was conducted to examine the anatomical features of some woody species in relation to their adaptation to the environment.

Materials and methods

Leaf anatomical study was conducted on the following woody species: *Azadirachta indica* (*Meliaceae*), *Capparis erythrocarpa* (*Capparaceae*), *Millettia thonningii* (*Fabaceae*), *Chromolaena odorata* (*Asteraceae*), *Zanthoxylum zanthoxyloides* (*Rutaceae*), *Griffonia simplicifolia* (*Fabaceae*), *Lonchocarpus macrophyllus* (*Fabaceae*), *Ficus capensis* (*Moraceae*), *Nauclea latifolia* (*Rubiaceae*) and *Morinda lucida* (*Rubiaceae*).

Anatomical Studies. The work was done in Accra in the coastal Savanna zone of Ghana from mid October to mid February 2001. Leaf epidermal strips of some of the species listed above, were obtained and mounted overnight in Canada balsam. Leaves of some of the species were collected and stored in Formal Acetic Alcohol (FAA) in sample tubes for subsequent anatomical sectioning in the laboratory using a microtome.

Thin Transverse sections (TS) of 3 µm thickness of each available leaf sample were made by holding the leaf in place in the correct orientation in a fine slit down a piece of carrot tuber and sectioning with the microtome. The thin sections of each sample obtained using the microtome, were then screened under a microscope, stained with safranin, washed through a series of ethanol of the following concentrations and sequence: 50% (1 min), 70% (1 min), 90% (1 min) and absolute ethanol (two changes 5 min each). The specimens were next stained in light green (½ - 1 min) and then cleared and washed in clove oil (5 min). Any over staining with light green was corrected by washing the specimens back through the series of alcohols before restaining in safranin. The sections were finally mounted in Canada balsam.

Results and discussions

Anatomical Studies. Anatomical studies presented here included camera lucida drawings of transverse section (TS) of lamina without the midvein and lower epidermal strips of leaves.

Azadirachta indica

Numerous stomata occurred on lower epidermis of *Azadirachta indica*. The epidermis is relatively thin. The palisade mesophyll composed of two layers of elongated closely packed cells. The spongy mesophyll is composed of loosely arranged cells with air spaces (Figs. 1a, 1b).

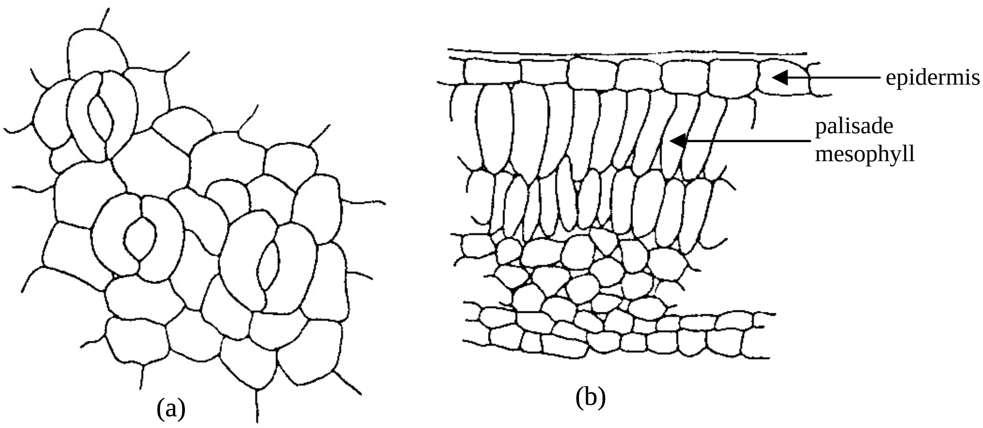

Fig. 1. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Azadirachta indica*

Millettia thonningii

Leaves are compound pinnate; pinnate entire with no hairs. Stomata confined to lower leaf surface. Epidermis is thick. Palisade mesophyll composed of two layers of longitudinal cells. Spongy mesophyll, loosely arranged with numerous air spaces.

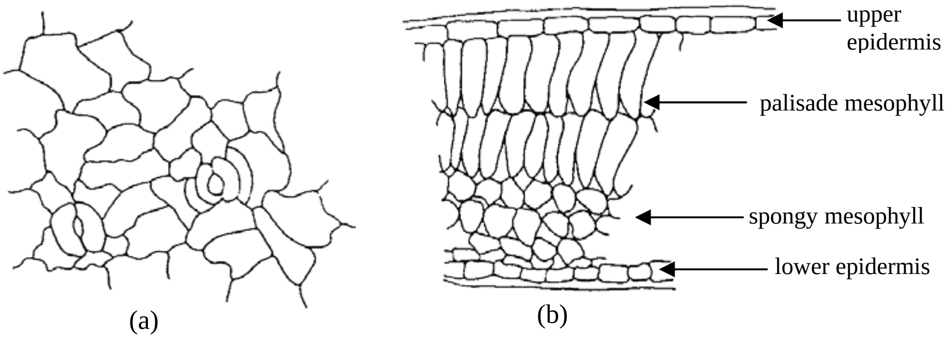

Fig. 2. (a) Camera Lucida drawing of lower epidermis and (b)TS of leaf lamina of *Millettia thonningii*

Capparis erythrocarpa

The leaf is simple, entire, leathery and evergreen. The plant is shrubby and thorny. Anatomically, there is a thick cuticle with no distinct epidermis (Fig. 3). The mesophyll is diffused with no clearly distinct palisade and spongy mesophylls. However, palisade mesophyll-like layer showed smaller cells than the spongy mesophyll-like layer. The spongy mesophyll-like layer had few air spaces (Fig. 3b). No distinct palisade layer evident. Cuticle appears to be prominent on both the upper and lower sides of leaf lamina.

Stomata appeared on only the abaxial leaf surface although YANNEY-WILSON (1963) recorded stomata on both surfaces (Fig. 3a).

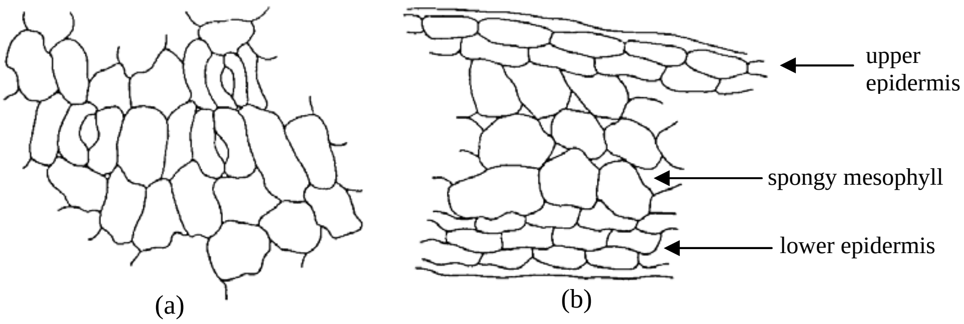

Fig. 3. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Capparis erythrocarpa*

Zanthoxylum zanthoxyloides

Leaves are compound pinnate, alternate with thorns on the lower surface of midvein and on the stem. The pinnae are entire and evergreen. Leaves are leathery with shiny upper surface. No hairs present. Mucilage gland occurred in the upper epidermis of the leaf (Figs. 4a, b).

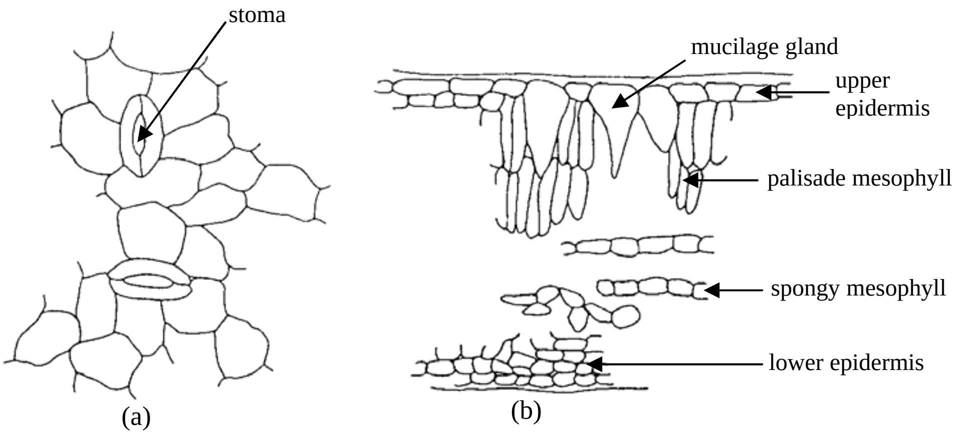

Fig. 4. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Zanthoxylum zanthoxyloides*

Griffonia simplicifolia

The leaves are simple and entire with folded margins. The leaves are leathery with shiny surface. There are no hair(s) present. Stomata appear on lower surface of leaf (Fig. 5a). Anatomy of the lamina showed a thick epidermis. Palisade mesophyll made up of two layers of short, closely packed palisade parenchyma cells; spongy mesophyll made up of loosely packed parenchyma cells with intercellular air spaces (Fig. 5b).

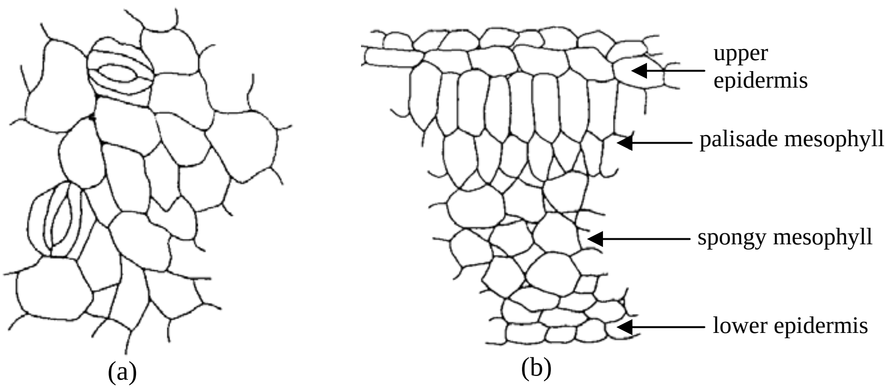

Fig. 5. (a) Camera Lucida drawing of lower epidermis and (b)TS of leaf lamina of *Griffonia simplicifolia* showing stomata (H.P)

Nauclea latifolia

The leaves are broad, entire, thick leathery with shiny adaxial surfaces. The surface is smooth.

Stomata occurred on the abaxial leaf surface. No stomata occurred on the adaxial leaf surface (Fig. 6a). The epidermis is thick with hypodermis. Palisade mesophyll cells are made up of two layers of elongated, closely packed cells (Fig. 6b).

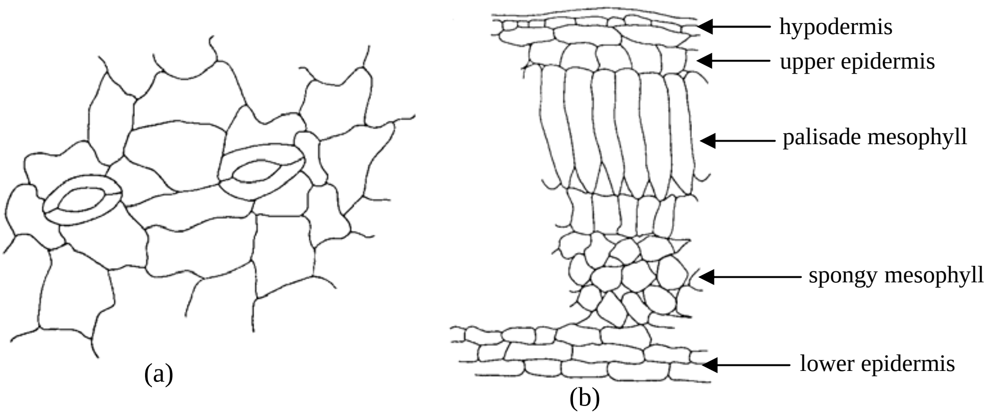

Fig. 6. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Nauclea latifolia* showing stomata (H.P.)

Ficus capensis

The Leaf is simple and entire, thick and pale green. There is presence of few unicellular hairs on the lower surface of leaf (Fig. 7b). Stomata confined to abaxial leaf surface (Fig. 7a). Epidermis contains numerous cystoliths (Fig.7a). Upper epidermis is thick. Palisade tissue composed of two layers of closely packed cells. Spongy mesophyll occupied larger portion of lamina with numerous intercellular air spaces (Fig. 7b).

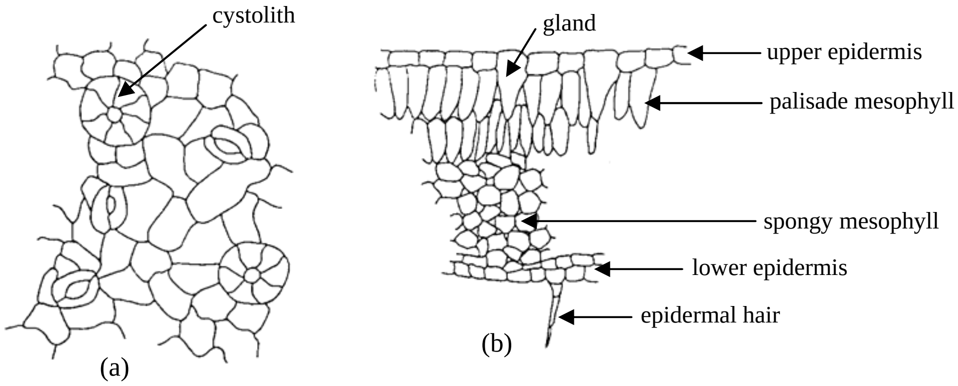

Fig. 7. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Ficus capensis* (H.P.)

Lonchocarpus macrophyllus

Epidermis composed of one layer of cell thick. Palisade mesophyll composed of two layers of longitudinal closely packed cells. The spongy mesophyll cells loosely arranged with numerous intercellular air spaces (Figs. 8a, b).

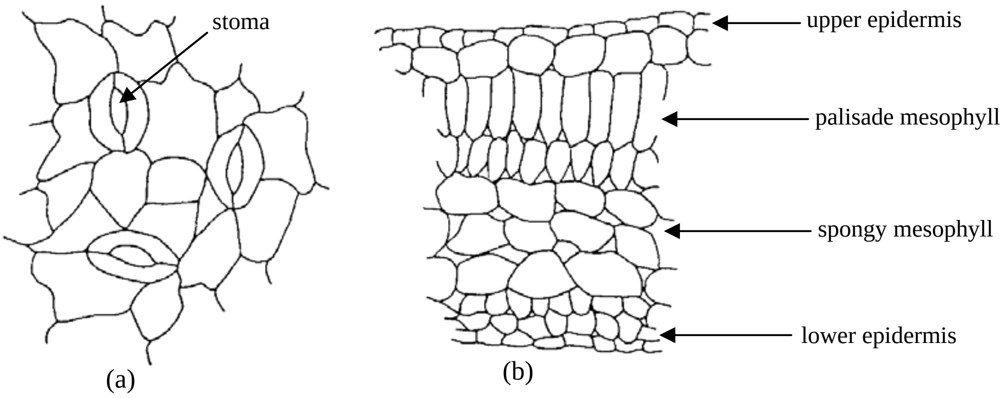

Fig. 8. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Lonchocarpus macrophyllus*

Morinda lucida

Leaves are pinnate; pinnae entire and leathery. Few epidermal hairs present on the lower epidermis (Fig. 9a). Stomata occurred on the lower epidermis. Epidermis composed of two layers of cells. Palisade mesophyll, one layer thick. Spongy mesophyll composed of several layers of cells (Fig. 9b).

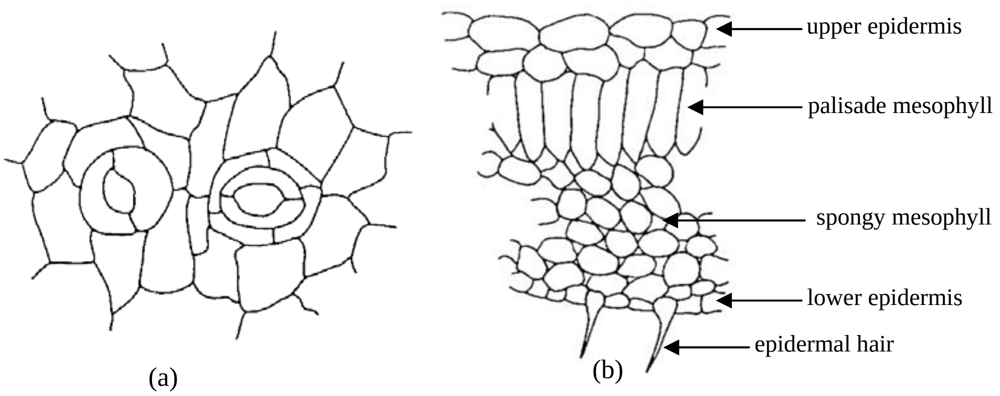

Fig. 9. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Morinda lucida*

Chromolaena odorata

Stomata confined to the lower epidermis. Epidermal cell are very large with undulating cell walls. Leaf anatomy showed two layers of short cells palisade mesophyll. Spongy mesophyll composed of loosely packed cells (Figs. 10a, b).

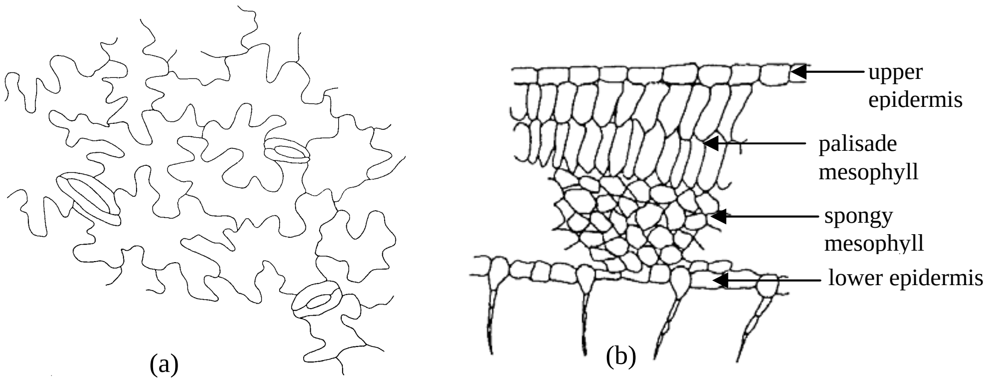

Fig. 10. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Chromolaena odorata* (H.P.)

Stomata Distribution/Frequency and Dimensions

The stomatal frequency, stomatal length and breadth (at the widest portion of stoma) are shown in Table 1.

Tab. 1. Diurnal Stomatal Dimensions of Some Species in Study Area at Pinkwae (Values are Means of Five Replicate Fields of View)

Species	**Stomatal frequency (L.P.)**	**Stomatal length (µm)**		**Stomatal width (µm)**	
		Morning	Afternoon	Morning	Afternoon
Azadirachta indica	1137 ± 27	0.028	0.028	0.014	0.014
Capparis erythrocarpa	484 ± 4	0.014	0.014	0.007	0.007
Chromolaena odorata	341 ± 0	0.028	0.028	0.014	0.007
Griffonia simplicifolia	486 ± 5	0.021	0.014	0.014	0.007
Millettia thonningii	512 ± 0	0.014	0.014	0.007	0.007
Morinda lucida	617 ± 0	0.028	0.028	0.014	0.007
Nauclea latifolia	607 ± 6	0.028	0.028	0.007	0.007
Zanthoxylum zanthoxyloides	452 ± 6	0.028	0.028	0.007	0.007

L.P. Low Power Magnification (10 x 10 x 10)

The stomatal frequency of *Azadirachta indica* was very high. With the exception of *Malachantta alnifolia* and *Chromolaena odorata* the stomatal frequencies of the species were relatively high.

The stomatal dimensions showed that most of the species maintained constant stomatal length during the study period except *Griffonia simplicifolia* that increased the stomatal width during the afternoon. With the exception of *Morinda lucida*, *Griffonia simplicifolia* and *Chromolaena odorata*, which showed reduction in the breadth of stomata, the other species maintained constant stomatal width.

The anatomical features of *Azadirachta indica*, showed relatively thick cuticle, with a double layer of palisade mesophyll (Fig. 1), which may probably be the feature for its adaptation to withstand the drought stress. Also, the small pinnae area, high stomatal frequency (Tab. 1) coupled with open stomata may have contributed to the efficiency of the plant; as these features have been reported to influence high diffusion conductance [MOONEY & GULMON, 1979; BANNISTER, 1978]. In their reports it was indicated that high transpiration rates occur when leaf resistance were low; and that was dependent on stomatal depth, area and number. BANNISTER (1978) also indicated that leaf modification was associated with dry habitats; and that epidermal modification might serve to maintain epidermal turgidity and thus enable the stomata to remain open [BANNISTER, 1964]. The high transpiration rates may also be effective in cooling the leaves.

Morphological features have been reported to be features used by plants to withstand drought [EHLERINGER & MOONEY, 1978; MOONEY & al. 1977; MULROY, 1979; TURNER, 1986]. TURNER (1986) indicated leaf wilting and leaf rolling to increase water use efficiency in rice. These features were reported to increase the stomata depth. BANNISTER (1964) observed hairiness in *Calluna* to support the epidermal modification that enables the stomata to remain open over a wide range of water deficits. In *Nauclea latifolia* the cuticle was relatively thin. The species may probably be using the leaf pubescence and the peculiar anatomical features to withstand drought.

All the species studied showed relatively thick cuticle and this may probably have contributed to their ability to withstand drought stress. However, *Zanthoxylum zanthoxyloides* showed the presence of mucilaginous cells in the epidermis which may possibly have contributed to its ability to tolerate the drought stress. It is reported that mucilage can absorb water and hence form a loose gel to help plants withstand drought [GREEN & al. 1986]. *Capparis erythrocarpa* showed the cuticle penetrating between epidermal cells. The spongy mesophyll was also relatively closely packed which may also contribute adaptation to drought.

The features observed conform to those observed and reported by YANNEY-WILSON (1963). Hairiness has been reported to be more likely concerned with protection from excessive insolation than from high transpiration [YANNEY-WILSON, 1963; SCHULZE & al. 1987].

With the exception of *Chromolaena odorata*, *Griffonia simplicifolia* and *Morinda lucida* which or that reduced their stomatal breadth in the afternoon, all the other species showed constant breadth/width dimensions. Also *Griffonia simplicifolia* also showed reduction in stomatal length in the afternoon. It is believed that during stomatal movement (opening and closure), the stomatal length is fixed but there is variation in the width of the stoma [MOONEY & GULMON, 1979; GIFFORD & MUSGRAVE, 1973]. YANNEY-WILSON (1963) however, reported reduction in the stomatal aperture of *Capparis erythrocarpa* during the afternoon. *Capparis erythrocarpa* was reported in the work of YANNEY-WILSON (1963) as the species with the thickest cuticle, however, in this study *Nauclea latifolia* showed the thickest cuticle.

Conclusions

The results showed that *Azadirachta indica* was a good potential in plant-water economy. *Millettia thonningii* on the other hand is drought-resistant deciduous. The study revealed variation in the stomatal shapes. The leaf anatomy of the plant studied were varied and species specific. However, double layer of palisade mesophyll was very common. This could be a characteristic for adaptation to the stress environment. The stomatal dimensions of the species with time of day showed that all the plants had adaptation mechanisms the stress environment. *Azadirachta indica* with numerous stomatal compared to the leaf surface remained evergreen during the dry season. It could be using an innate ability to adapt to the drought stress. However, leaf anatomy, stomatal behavior coupled with external morphological features may be contributing to the efficiency of the species.

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