

A Comparative Analysis of the Stomatal Density of *Ficus septica* Burm. f. Along an Altitudinal Gradient at the Kalungan Forest of Mt. Talinis, Bacong, Negros Oriental

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ABSTRACT

Altitude is one of the environmental factors that affects a number of plant developmental processes, including stomatal development. In this study, the nail polish imprinting method was used to determine if stomatal density increases with increasing elevation in *Ficus septica* Burm. f. a widely distributed woody species in the Kalungan Forest, Mt. Talinis, Bacong, Negros Oriental. Sampling was done from 392-894 m above sea level from three trees per elevation. Six elevation stations were identified at 100 m interval. Leaf stomatal imprints from three trees per elevation were obtained. Stomata were counted manually with the aid of software ImageJ 1.45. Stomatal density values of the highest and lowest elevation were $3\ 869.6 \pm 814.1$ and $1\ 125.6 \pm 357.1$, respectively. Regression analysis showed a strongly linear relationship between stomatal density and elevation.

Keywords: fig tree, elevation, stomata, Moraceae, Philippines

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INTRODUCTION

Ficus septica Burm. f. is a member of the order Rosales, family Moraceae and are collectively known as 'fig trees'. It is commonly known as 'hauili' (Merrill, 1923) but it is locally called 'labnog' in Negros Oriental. It is one of the most common non-cultivated woody plants in Mt. Talinis (Heidemann and Utzurrum, 2003). Bats commonly feed on its fruits or "figs" which could be one reason for its wide distribution in the area (A. Carino, pers. Comm., 28 April). This tree is indigenous to Indo-Pacific countries, including the Philippines where it is distributed in thickets at low to medium altitudes (Lanting and Palaypayon, 2002). Its leaves are hypostomatic and its stomates are paracytic (Sonibare *et al.*, 1995; Klimko and Truchan, 2006).

Stomata are minute pores that are mostly found on the lower epidermis of leaves and are regulated by guard cells (Nabors, 2005). Entry of carbon dioxide for glucose synthesis and the escape of water and free oxygen happen when the stomata are open (Swarthout *et al.*, 2011). Different plant species vary in terms of stomatal density based on their specific regulatory needs. Since stomata are so responsive, their behavior is a good focus for study (Taiz and Zeiger, 2002).

Previous studies (i.e., Körner and Cochrane, 1985; Körner *et al.*, 1986; Woodward, 1986; Hovenden and Brodribb, 2000; Woodward *et al.*, 2002) have shown a positive relationship between stomatal density and elevation with other plant species. According to Körner *et al.* (1986), Hultine and Marshall (2000), Greenwood *et al.* (2003), Qiang *et al.* (2003), this phenomenon does not apply to all species and all localities.

Results of some of the more current studies corroborate with findings that stomatal density increases with altitude (*e.g.*, McElwain, 2004; Kouwenberg, 2007, Qiang *et al.*, 2003). Other researchers, however, showed the negative relationship between stomatal density and altitude (Hultine and Marshall, 2000). For example, Johnson *et al.* (2009) posited that although the partial pressure of carbon dioxide decreases with altitude, the diffusion of gas phase molecules such as CO₂ increases at lower atmospheric pressure. This position suggests that at greater altitudes, the photosynthetic carbon dioxide does not appear to be rate-limited due to the compensatory effect of increased CO₂ diffusion rates. Stomatal CO₂ partial pressure in turn will not cause a change in stomatal density of plant species in areas of higher altitudes. Numerous studies like that of Hovenden and Brodribb (2000) have shown an increase in stomatal density with elevation. Qiang *et al.* (2003) however, suggested that carbon dioxide is not a primary factor that affects the stomatal density.

McElwain (2004) suggested that the findings of Johnson *et al.* (2009) showing inverse relationship between stomatal density and altitude is due to their failure to consider the fact that transpiration rates are often extremely elevated at high elevation due to higher light intensity, increased diffusion of water in air at reduced atmospheric pressure. According to Gale (2004), an increased density gradient of water vapors out of the leaf into the ambient air. In addition, although abiotic factors do not directly influence the absolute number of stomata, it can influence the size and spacing of stomata resulting to high or low densities (McElwain, 2004). At higher elevations, evaporative demand is not excessively high because of lower temperature; therefore, stomatal density varies greatly depending on the current ecophysiological understanding of plant growth along elevation gradients. Still, when evaporative demand is not high, stomatal density variation is not expected as stomatal response to declining CO₂ partial pressure is not needed (Kouwenberg *et al.*, 2007).

Various experiments have been conducted highlighting a new paleoaltimetry method that uses leaf stomatal density response to pressure, that increases with altitude and vice versa (*e.g.*, Haworth *et al.*, 2009; Ilgin and Caglar, 2009; Kouwenberg *et al.*, 2007; McElwain, 2004; Beerling and Chaloner, 1993). These studies have deliberated about the stomatal density and its response to different environmental factors such as atmospheric pressure, carbon dioxide concentrations, and altitude.

To date, explanations for the functional relationship between plant leaf stomatal density and elevation are still debated by plant ecophysiologicals (*e.g.* Friend and Woodward, 1990; Gale, 1972, 2004; Smith and Donahue, 1991; Terashima *et al.*, 1995; Woodward and Bazzaz, 1988). Furthermore, whether stomatal density increases or decreases with altitude, knowledge of stomatal density's response to altitude can evaluate certain plant species' reactions to different geographical conditions (Aslantas and Karakurt, 2009).

In order to address the hypothesis that stomatal density increases with altitude, the nail polish imprinting method was used to accurately count the number of stomata within an area of 5mm² per leaf (Case, 2004; Solangi *et al.*, 2010) and compare the densities of *F. septica* leaves from altitudes 300-900 meters above sea level.

MATERIALS AND METHODS

Site and field sampling

Leaf samples were collected from Kalungan Forest of Mt. Talinis, Bacong, Negros Oriental on May 5, 2012 (Figure 1). The forest is part of Mt. Talinis (the foot of Cuernos de Negros) which stands at 900 meters above sea level. The peak of Mt. Talinis is at 1,900 meters above sea level. Bacong (9° 14' 45" North, 123° 17' 41" East) is a municipality adjacent to Valencia and is 9.4 km away from the province's capital Dumaguete City.

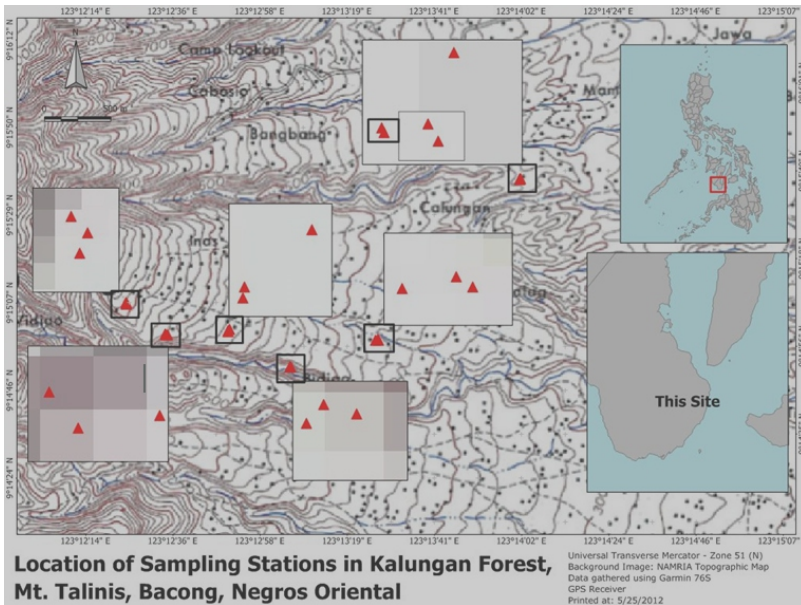


Figure 1. Terrain image of all the elevation stations at Kalungan Forest of Mt. Talinis, Bacong Negros Oriental

Six elevation stations were sampled. Each elevation station was composed of three trees. Geographical coordinates of the elevation stations (Table 1) were read using the 76 GPS Receiver Unit and was plotted in Manifold 8.0. The sampling stations were approximately 900 m above sea level with 100 m decrement down to 300 m above sea level. Each sampling station was described to have different biotic compositions (Table 1).

Table 1. The sampling station description at the Kalungan Forest of Mt. Talinis, Bacong, Negros Oriental

Station no.	Elevation (m)	Description
1-3	894.4-887.7	Primarily a grassland composed of few trees like <i>F. septica</i>
4-6	794.4-788.0	Within the forest patch with mixed agricultural vegetation and dominated by <i>F. septica</i>
7-9	694.4-689.2	A grassland alongside a sunflower patch with few mature trees
10-12	589.9-588.7	A road surrounded by flowering plants mostly <i>Helianthus</i> sp.
13-15	497.1-496.6	A road with shrubs and ferns growing alongside.
16-18	391.7-388.1	A man-made forest at Liptong Woodlands.

Leaf Sampling

For each elevation station, top ten, mature fully expanded green leaves (Woodward *et al.*, 2002; Kofidis and Bosabalidis, 2008) of identical sizes were obtained according to the method of Ilgan (2009) with the assumption that they had developed in full irradiance (Figure 2). Leaves were randomly gathered from different branches from each of the three trees. Each leaf sample was treated using the nail polish imprinting method (Case, 2004; Solangi *et al.*, 2010).

The nail polish imprinting method was used to obtain the stomatal imprint. A thin layer of natural nail polish (Bobbie™) was applied to a 5mm² rectangular strip approximately between the 2nd and the 3rd vein on the abaxial part of the leaf considering that stomata of *Ficus* species are hypostomatic (Solangi *et al.*, 2010). When the nail polish had dried up, the stomatal imprint was slowly pulled away and was immediately mounted on a glass slide. The imprint was then viewed under the light microscope at 40x magnification (Solangi *et al.*, 2010). The image under the microscope was captured using a digital camera and was uploaded to the computer. Using the cell counter option of the software ImageJ 1.45, the number of stomata was counted. The total stomatal count was averaged for every tree per elevation. The trend was shown using a linear regression to view the relationship between stomatal density and elevation.

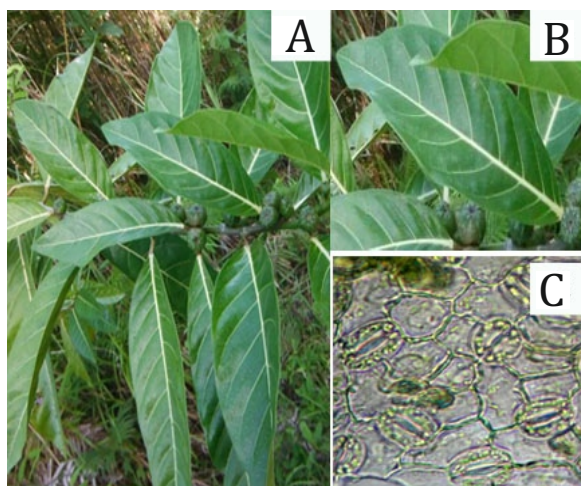


Figure 2. *Ficus septica* Burm. f. (A) branch (B) mature leaf (C) abaxial stomata - Magnification x400.

RESULTS AND DISCUSSION

The stomatal counts of the 10 leaves from each of the three trees from all six elevations were averaged. The mean stomatal densities from the three trees of highest elevation range, 894.3 – 892.9 meters above sea level, which are 3869.6, 3547.4 and 3568.2 were far significantly higher than the mean stomatal densities 1746.8, 1415.7 and 1125.6 from those that were from the lowest elevation range, 391.7-388.1 meters above sea level ($t=0.08$, $P= 0.93$, $n=17$). Every sampling station was composed of *F. septica* trees growing at a significant distance from each other. Thus, each of the three trees in each station was found in a different altitude. This is the reason why each of the station was described as within a range of elevation. Standard deviation for each sampling station was high due to a high variation among the stomatal counts (Figure 3).

The response of stomatal density to elevation was objectively linear (Fig. 4), as shown by the slope of the regression line. The computed correlation coefficient is approximately 0.92. It indicates a strong linear relationship between stomatal density and elevation.

Since its r -value was close to positive 1, there is significant proof that stomatal density and altitude are two interrelated variables. The value of $r^2 = 0.85$ indicates that approximately 85% of the variation in elevation may be explained by the stomatal density.

Comparative analysis of the stomatal density of *Ficus septica* Burm. f.

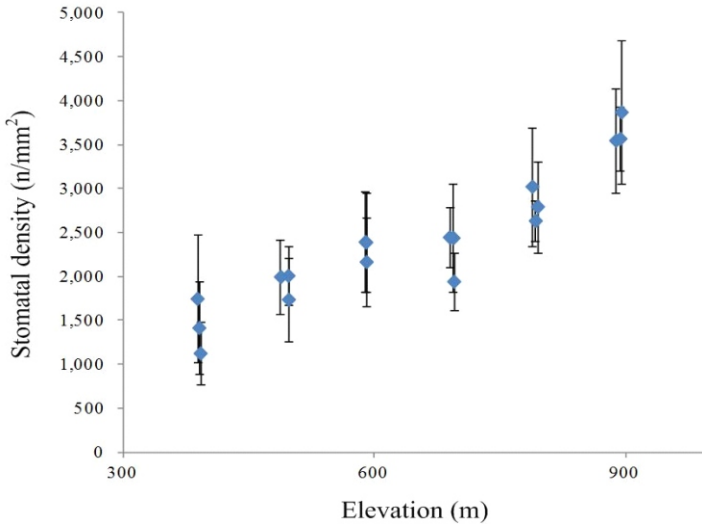


Figure 3. Mean ± stomatal density values of matured *Ficus septica* leaves collected randomly from each tree at the Kalungan Forest of Mt. Talinis, Bacong Negros Oriental along an altitudinal gradient (May 2012).

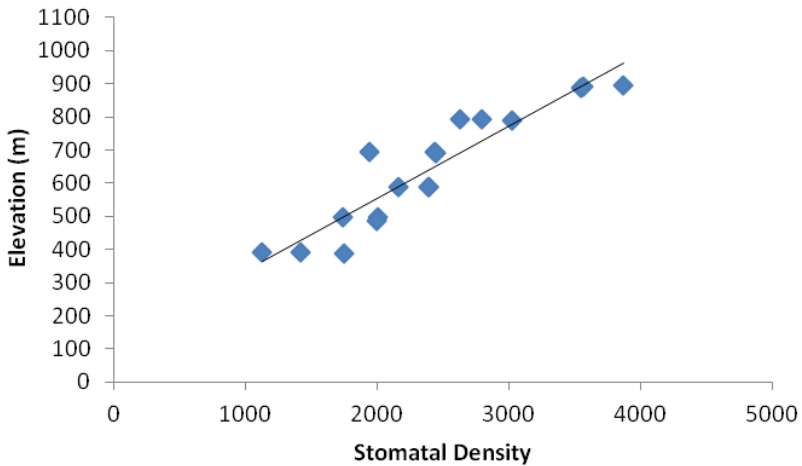


Figure 4. Relationship between stomatal density versus elevation of *F. septica* collected at the Kalungan Forest of Mt. Talinis, Negros Oriental. Squares indicate the SD mean of ten leaves per tree. SD for every leaf was averaged per elevation for regression analysis. It has a slope $y = 0.2187x + 116.13$, $R^2 = 0.8612$ and a p value = 0.936.

The extensive distribution of *F. septica* trees in Mt. Talinis makes it a practical subject for the study of species (Heideman and Utzurrum 2003). Stomatal count from 10 leaves of each of the three *F. septica* trees in all six elevation ranges have clearly shown that stomatal density increases with elevation.

At present, there are no published studies on *F. septica* showing that higher altitudes result in greater stomatal density. However, a study conducted by McElwain (2004) on California black oaks (*Quercus kelloggii*) concluded an increasing stomatal density with increasing elevation. In the mountains of Greece, stomatal density on the adaxial and abaxial surfaces of *Origanum vulgare* leaves were also shown to increase with increasing elevation (Kofidis *et al.*, 2003).

Likewise, in an experiment by Woodward and Bazzaz (1988) where plants were grown under lower air pressure but with constant CO₂ mixing ratios, stomatal densities increased significantly to maintain normal photosynthetic rates, even with lower CO₂ availability (Kouwenberg, 2007). Comparing results, CO₂ partial pressure may also be associated with increased stomatal densities in higher elevations, given that changes in CO₂ partial pressure significantly influenced the stomatal frequencies in the Woodward and Bazzaz (1988) experiment.

Along with increased elevation, other factors such as H₂O diffusion in air and the density gradient of H₂O vapors out of the leaf also increased. According to von Caemmerer and Farquhar (1981), at higher elevations, H₂O vapor efflux through the stomata hinders CO₂ diffusion into the leaf, causing the plant to increase its transpiration water vapor loss rates to compensate for the higher diffusion coefficient of CO₂ at lower pressure. Higher transpiration rates require increased stomatal density at high elevations to maintain adequate conductance to CO₂ diffusion (McElwain, 2004). Moreover, higher stomatal densities allow optimum photosynthetic activity when photosynthesis is not hindered by a number of several disadvantages on higher elevations such as harsh climatic conditions and lower temperature. These disadvantages limit the time of photosynthetic activity by shortening the growing season, a significant reason for the needed reparation done over increasing the maximum photosynthetic potential (Kouwenberg, 2007). All these may explain for the increased stomatal density of *F. septica* leaves in higher elevations. Another explanation is since barometric pressure decreases quite predictably with increasing elevation, there is a corresponding decrease in the partial pressure of CO₂ (pCO₂), mainly due to the fact that the molar fraction of CO₂ in the atmosphere does not change with elevation (McElwain, 2004).

The significantly ($p < 0.05$) high linear relation between elevation and stomatal density provides the prospective of reconstructing paleoelevations. Although increased stomatal density is not solely dependent on elevation, it is clear that the two variables are linked to each other (Kuowenberg, 2007).

On the perspective of developmental anatomy, stomatal density varies greatly in different parts of the plant and is influenced by environmental factors such as light and CO₂ levels (Evert, 2006). Bird and Gray (2003) suggested that light levels, atmospheric CO₂ concentration, humidity and drought signals have an effect on the level of stomatal development. It is unsurprising that stomatal numbers be sensitive to environmental conditions because stomata are linked to the rate of CO₂ uptake and fixation. Several levels of control over stomatal development include control over the spacing of individual cells to ensure that stomata are separated by at least one epidermal pavement cell, that there is control of stomatal numbers as part of tissue patterning during development, and that there is control of stomatal numbers by environmental conditions. These factors act during meristemoid initiation and can control the number of meristemoids formed in the epidermal cells. They are also capable of controlling the number of satellite stomatal complexes after the formation of primary anisocytic complex.

This study can provide clues as to the stomatal development of *F. septica* trees, including a glimpse of their transpiration and physiology, especially those that are cultivated in higher elevations. Future research studies can focus on stomatal index or stomatal size, for instances, to be able to really associate stomatal development with its geographical aspects.

CONCLUSION

The findings of this research have shown that there is direct relationship between stomatal density and elevation. There is an increasing stomatal density of *Ficus septica* leaves gathered from the Kalungan Forest, Mt. Talinis, Bacong Negros Oriental at 300 meters up to 900 meters above sea level supported by statistical analysis showing a significantly high value for the multiple correlation coefficient. The results imply that high stomatal density of *Ficus septica* leaves at high elevations may be an adaptation to certain environmental factors that are related to elevation such as pressure, CO₂ concentration, and transpiration rates.

REFERENCES

- ASLANTAS R. and H. KARAKURT. 2009. *The effects of altitude on stomata number and some vegetative growth parameters of some apple cultivars*. Research Journal of Agricultural and Biological Sciences **5(5)**: 853-857.
- BEERLING, D. J. and W.G. CHALONER. 1993. *The impact of atmospheric CO₂ and temperature change on stomatal density: observations from Quercus robur Lammas leaves*. Annals of Botany **71**: 231-235.
- BIRD, S.M. and J.E. GRAY. 2003. *Signals from the cuticle affect epidermal cell differentiation*. New Phytologist **157**: 9-23.
- CASE, S. 2004. *Leaf stomata as bioindicators of environmental change*. Access Excellence Exchange: Fellows' Collection, [2012 March 28] Available from: <www.accessexcellence.org/AE/AEC/AEF/1994/case_leaf.html>.
- EVERT, R.F. 2006. *Esau's Plant anatomy : meristems, cells, and tissues of the plant body : their structure, function, and development*. John Wiley & Sons, Inc., Hoboken, New Jersey, 3rd ed. 220p.
- FARQUHAR, G. D. 1981. *Carbon isotope discrimination by plants and the ratio of intercellular and atmospheric CO₂ concentrations in: Pearman GI Carbon dioxide and climate*. Australian Academy of Science **1**:105-110.
- FRIEND, A.D. and F.I. WOODWARD. 1990. *Evolutionary and ecophysiological responses of mountain plants to the growing season environment*. Advances in Ecological Research. **20**: 59-124.
- GALE, J., 1972. *Elevation and transpiration: Some theoretical considerations with special reference to mediterranean-type climate*. Journal of Applied Ecology **9**:691-701.
- GALE, J., 2004. *Plants and altitude—Revisited*: Annals of Botany. **94**: 199.

- HEIDEMANN P.D. and R.C.B. UTZURRUM. 2003. *Seasonality and synchrony of reproduction of three nectarivorous Philippine bats*. BMC Ecology 3:11.
- HOVENDEN, M.J. and T. BRODBIBB. 2000. *Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in Southern Beech, Nothofagus cunninghamii*. Australian Journal of Plant Physiology 27:451–456.
- HULTINE, K.R. and J.D. MARSHALL. 2000. *Altitude trends in conifer leaf morphology and stable isotope composition*. Oecologia. 123:32–40.
- ILGIN, M. and M. CAGLAR. 2009. *Comparison of leaf stomatal feature in some local and foreign apricot (Prunus armeniaca L.) genotypes*. African Journal of Biotechnology 8 (6):1074–1077.
- JOHNSON, D., W. SMITH, and M. SALMAN. 2009. *Climate- independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO₂ partial pressure: Comment and Reply*: e82-e83.
- KLIMKO, M. and M. TRUCHAN. 2006. *Morphological variability of the leaf epidermis in selected taxa of the genus Ficus L. (Moraceae) and its taxonomical implications*. Acta Societatis Botanicorum Poloniae. 75(4):309-324.
- KOFIDIS G. and A.M. BOSABALIDIS. 2008. *Effects of altitude and season on glandular hairs and leaf structural traits of Nepeta nuda L.* Botanical Studies 49: 363-372.
- KÖRNER, C. and P.M. COCHRANE. 1985. *Stomatal responses and water relations of Eucalyptus pauciflora in summer along an elevational gradient*. Oecologia 66:433–455.
- KOUWENBERG, L., W. KURSCHNER, and J. McELWAIN. 2007. *Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry*. Reviews in Minerology & Geochemistry 66:215-241.

- HAWORTH, M., J. HEATH, and J. McEWAN. 2009. *Differences in the response of stomatal index to atmosphere CO₂ among four genera of Cupressaceous conifers*. *Annals of Botany* **105**(1): 411-418.
- LANTING JR., M.V., and C.M. PALAYPAYON. 2002. *Forest tree species with medical uses*. Department of Environmental and Natural Resources. **11**: 1-23.
- McELWAIN, J.C. 2004. *Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO₂ partial pressure*. *Geology* **32**:1017-1020.
- MERRILL, E. *An enumeration of Philippine flowering plant* [Internet]. Biodiversity Heritage Library: Manila, Bureau of Printing; 1923 [cited 2008 March 28]. Available from: <<http://www.biodiversitylibrary.org>>.
- NABORS, M. 2005. *Botany An Introductory Approach*. Singapore: Pearson Education South Asia Pte Ltd. 659 p.
- PETERSON, K., A. RYCHEL, and K.TORII. 2010. *Out of the mouths of plants: The molecular basis of evolution and diversity of stomatal development*. *The Plant Cell*. **22**: 296-306.
- QIANG, W., X. WANG, T. CHEN, L. FENG, Y. HE, and G. WANG. 2003. *Variations of stomatal density and carbon isotope values of Picea crassifolia at different altitudes in the Qilian Mountains*. *Trees* **17**:258-262.
- SMITH, W.K. and R. DONAHUE. 1991. *Simulated effect of latitude on photosynthetic CO₂ uptake potential in plants*. *Plant, Cell and Environment* **14**: 133-136.
- SOLANGI, A.H., M.A. ARAIN, and M.Z. IQBAL. 2010. *Stomatal studies of coconut (Cocos nucifera L.) varieties at coastal area of Pakistan*. *Journal of Botany* **42**(5): 3015-3021.
- SONIBARE, M.A., JAYEOLA, A.A., EGUNYOMI, A., MURULA, J. 2005. *A survey of epidermal morphology in Ficus Linn. (Moraceae) of Nigeria*. *Bot. Bull. Acad.Sinica*. **46**: 231-238

- TAIZ, L. and E. ZEIGER. 2002. *Plant Physiology*. Sinauer Associates; 3rd edition. 690 p.
- TERASHIMA, I., T. MASUZAWA, H. OHBA, and Y. YOKOI. 1995. *Is photosynthesis suppressed at higher elevations due to low CO₂ pressure*. *Ecology* 76: 2663–2668.
- VON CAEMMERER, S. and G.D. FARQUHAR. 1981. *Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves*. *Planta* 153: 376–387.
- WOODWARD, F.I. and BAZZAZ, F.A. 1988. *The responses of stomatal density to CO₂ partial-pressure*. *Journal of Experimental Botany* 39:1771–1781.
- WOODWARD, F.I., J. A. LAKE and W.P. QUICK. 2002. *Stomatal development and CO₂: ecological consequences*. *New Phytologist* 153: 477–484.