Variation in Some Structural, Biochemical and Gas Exchange Characteristics of Sun and Shade Leaves of Vernonia amygdalina

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Abstract--- *Light intensity has an influence on the photosynthetic capacity of a plant. The objective of this research was to investigate the variation in some structural, biochemical and gas exchange characteristics of sun and shade leaves of Vernonia amygdalina. Sun and shadeleaves were studied, and theirlight response parameters including light saturation point (LSP), light compensation point (LCP) and apparent quantum yield were estimated. Photosynthetic pigment were quantified spectrophotometrically. The stomatal density was determined using the nail polish method, and examined using light microscope. The result of this study proves that high light intensity influence gas exchange, stomatal density, leaf thickness and photosynthetic pigment of the studied plant. As the light intensity increases,* A_{max}*, LSP and LCP increases. There are significant differences between the light response characteristics, photosynthetic pigment and stomatal density of sun and shadeleaves (*$P<0.05$*). Statistically significant negative correlation (*$P<0.05$*) was achieved among stomatal density and transpiration rate(E). As stomatal density increases, E decreases. The result leads to a conclusion that sun leaves of Vernonia amygdalina contribute the highest assimilation rate to the plant than shade leaves. Also, the higher stomatal density of sun leaves provides water saving to the plant.*

Keywords--- *Gasexchange, Light Response Characteristics, Shade Leaves, Stomatal Density, Sun Leaves, Vernonia Amygdalina.*

I. INTRODUCTION

Vernonia amygdalina; family *Asteraceae* is also known as bitter leaf. It is a small shrub which can be consume as food or as a drug. The plant is native to tropical region especially Africa. It is also widespread in peninsular Malaysia. The specie is mostly use for traditional medicine. The plant extracts is having anti-cancer and antioxidant effect. It can enhance chemotherapy and cause cell apoptosis. It is also having anthelminthic effect.Previous studies on *Vernonia amygdalina* were mostly related to its medicinal effect, ethno pharmacology (State, 2016) (Edgar, 2014)(Ogunlana, 2016).

The natural environment does not have a stable condition, it always changes, and these changes can lead to various effects in the structural, biochemical and gas exchange characteristics of a leaf including its shape, curling

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degree, and its surface characteristics. One of the most important environmental factor affecting plants is light(Pengelly et al., 2010). Light affects the growth and development of plants by influencing the morphology of a single leaf as well as the morphology of the whole plant. Due to the fact that adaptation leads to survival, plants can adapt to varying light intensity depending on their environmental light availability. This adaptation would be possible if plantchanges the distribution of its biomass and its morphology, in order to be able to utilize the amount of light they receive, so as to survive(Xue, Li, Zhu, Lin, & Wang, 2011).

Leafphotosynthesis, pigment contents as well as secondary metabolites production can be affected by the changes in the amount of light intensity a plant is receiving. When the light intensity is low, the light received is weak and therefore the plant will produce more photosynthetic pigments, more especially chlorophyll (Chl) b, leading to reduction of Chl a/b ratio as well as reduction in the net assimilation rate (A_{net})(Liu, Guo, Wang, & Ouyang, 2002). When the plant receives high light intensity, photoinhibition will occur because the excess photons would not be utilized in photosynthesis (Shirke & Pathre, 2003), leading to oxidative stress due to the large amount of reactive oxygen species (ROS). For example, *Camptothecaacuminuita* grown at 75% irradiance have greater net photosynthesis rate than the plant grown under 100% irradiance(Ma et al., 2015). Partially shaded plants usually have lower values of stomatal conductance to water vapour (g_{sw}) than unshaded plants.

The objective of this study was to evaluate how varying light intensity affect the structural, biochemical and gas exchange characteristics of *Vernonia amygdalina*.

II. Materials And Methods

2.1 Plant material

Branches with fully developed sun and shade adapted leaves were sampled. Shade leaves at the inner tree crown received up to 100 µmol m^{-2} s^{-1}on sunny days, whereas the sun leaves were exposed to a maximum PPFD of about 1800 µmol m^{-2} s^{-1}. A branch with the desired leaf was cut from the tree and the cut endwas immediately re-cut under water to remove xylem embolisms. The branch end remained in the water during the measurements (K. Lichtenthaler, Alexander, Marek, Kalina, & Urban, 2007)

2.2 Gas exchange measurements

PPFD was measured using LI-6400 (LICOR, Lincoln, Nebraska, USA) portable photosynthesis system quantum sensor. Flow rate of pump was constant, leaf temperature was 30^{o}C while $CO_2$was constant (ambient). Net assimilation rate (A_{net}) was determined using open gas mode, using LI-6400. The youngest matured leaves of the plant (both light adapted and shade tolerant) were randomly sampled. PPFD was variable (2000, 1500, 1250, 1000, 750, 500, 250, 100, 50, 25, and 0 µmol m^{-2} s^{-1}). Acclimation of each leaf was done so as to avoid photo inhibition during measurements. This procedure was accompanied by light response curve. Light response curve was fitted according to(Marshal & Biscoe, 1980)and the Light response curve parameters were estimated using equation 1-3.

$$A_{net} = \frac{\phi PPFD + \sqrt{(\phi PPFD + A_{max})^2 - 4\theta\phi PPFD + A_{max}}}{2\theta} - R_d \quad (1)$$

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$$\text{LCP} = \frac{\theta \times (R_d)^2 - R_d \times A_{\max}}{(R_d \times \phi) - (\phi \times A_{\max})} \quad (2)$$

$$\text{LSP} = \frac{R_d + 0.9 \times A_{\max}) \times A_{\max} - \theta \times (R_d + 0.9 \times A_{\max})}{\phi(A_{\max} - R_d)} \quad (3)$$

Where A_{net} is the net photosynthetic rates (μmol CO2 m^{-2} s^{-1}),

A_{max} is the maximum photosynthetic rates (μmol CO2 m^{-2} s^{-1}),

LCP is the light condensation point (μmol CO2 m^{-2} s^{-1}),

LSP is the light saturation point calculated at 90 % of A_{max} (μmol CO2 m^{-2} s^{-1}),

ϕ is the apparent quantum yield (mol CO2 mol photons $^{-1}$),

R_d is daytime dark respiration rate (at no light; μmol CO2 m^{-2} s^{-1}), and

θ is curve convexity (dimensionless)

2.3 Determination of the photosynthetic pigments contents

The quantitative determination of the pigments presents in sun and shade leaves of *Vernonia amygdalina* was done using UV visible spectrometer according to (H. K. Lichtenthaler & Buschmann, 2005). The leaf used for net assimilation rate determination was harvested, and transferred immediately to the laboratory, weighed and grinded using mortar and 95 % ethanol. The suspension was centrifuged for 10 minutes at 4◦C, at 2000g. The procedure was done in the dark in order to avoid pigment loss. The supernatant was used for taking absorbance at 664.2 nm, 648.6 nm, and 470 nm respectively for Chl a, Chl b and carotenoids (Car) using a spectrophotometer. After taking the absorbance, the total content of the pigment was calculated using equation 4-6 and the resultant value was expressed in mg g^{-1} of fresh leaf weight.

$$\text{Chl a } (\mu g/ml) = 13.36\ A_{664.1} - 5.19\ A_{648.6} \quad (4)$$

$$\text{Chl b } (\mu g/ml) = 27.43\ A_{648.6} - 8.12\ A_{664.1} \quad (5)$$

$$\text{Car } (\mu g/ml) = (1000A_{470} - 2.13a - 97.64b) / 209 \quad (6)$$

Source: Lichtenthaler and Buschmann (2005)

2.4 Determination of leaf thickness and stomatal density

Leaf thickness was determined using the digital calliper while the stomatal density was determined using the fingernail polish impression (Xu & Zhou, 2008), and observed using a stereo microscope at 40X objective lens. The stomatal density (mm^{-2}) was calculated by dividing the area of field of view by the average number of stomata in a field of view.

2.5 Statistical analysis

All experiments were carried out in triplicates. Data was reported as the mean ± standard deviation, and all tests were performed using the IBM SPSS statistics 20.0 (New York: IBM Corp.). The data was tested for normality (Shapiro–Wilk normalitytest) before comparison of means. T-test was used to statistically analyse the data while

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Pearson correlation coefficient was used to evaluate the relationship between A_{net}, E, g_{sw} and stomatal density at a 95 % confidence level.

III. RESULTS AND DISCUSSION

3.1 Photosynthesis

The effect of varying light intensity on photosynthesis of *Vernonia amygdalina*is represented in figure 1. The light response curve was fitted based on non-rectangular hyperbola model. The curvature of the light response curve of the shadeleaves is acute while that of the sun leaves was gradual. The correlation coefficient (R^2) of the fitted curves were all greater than 0.996. The photosynthetic capacity of the plants is indicated by the light compensation point (LCP) and the light saturation point (LSP). The sun leaves are having greater photosynthesis capacity per area as shown in table 1. In both sun and shade leaves, A_{net} increases as the PPFD increased from 0-250 µmol m^{-2} s^{-1}) and then the assimilation increases slowly to a maximum level forming a light response curve. All photosynthesis parameters were higher in sun leaves than in shade leaves (table 1). LCP and LSP of sun leaves varied significantly from that of shade leaves ($P < 0.05$). The plant AQY did not differ significantly between the sun and shade leaves.

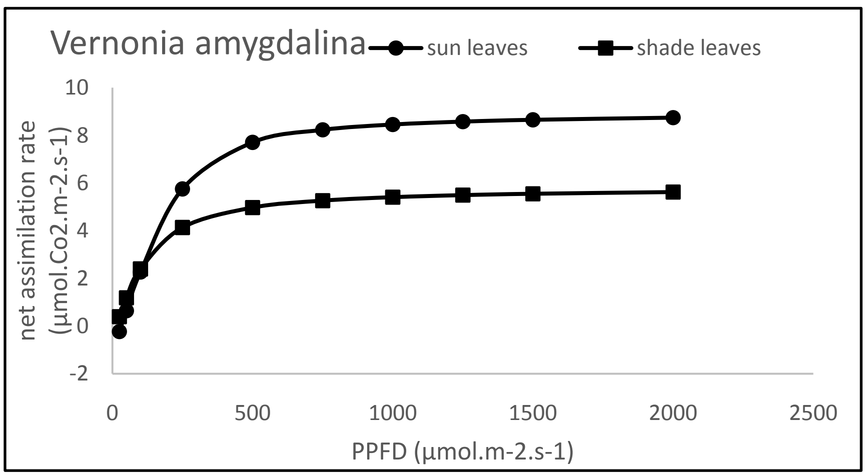

Figure 1: The photosynthetic light response curve of Vernonia amygdalina

Sun leaves usually differs from shade leaves by having a higher LSP. Sun leaves have higher A_{max} and lower curve curvature than shade leaves. Amax is higher in sun leaves because of their higher number of chloroplast per area, and their higher number of stroma exposed thylakoid membranes (Lambers, Chapin, & Pons, 2008).

The studied plant can grow best at high light intensity. A decrease in the light intensity leads to a decrease in A_{net}, E and g_{sw}. E and g_{sw}values varied significantly between sun and shade leaves. Shade leaves were having greater E than sun leaves ($P < 0.05$). A_{net} of sun leaves was significantly higher than that of shade leaves ($P < 0.05$).

Table 1: Light response characteristics of Vernonia amygdalina

	A_{max} (µmol.CO2 m-2 s-1)	LCP (µmol photons m-2 s-1)	LSP (µmol photons m-2 s-1)	AQY (mol CO2 mol-1 photons)
Sun leaves	10.10±0.46[a]	31.47±1.31 [a]	1014.33±72.57 [a]	0.039±0.007 [a]
shade leaves	6.33±0.55 [b]	13.90±2.26 [b]	704.67±56.58 [b]	0.036±0.009 [a]

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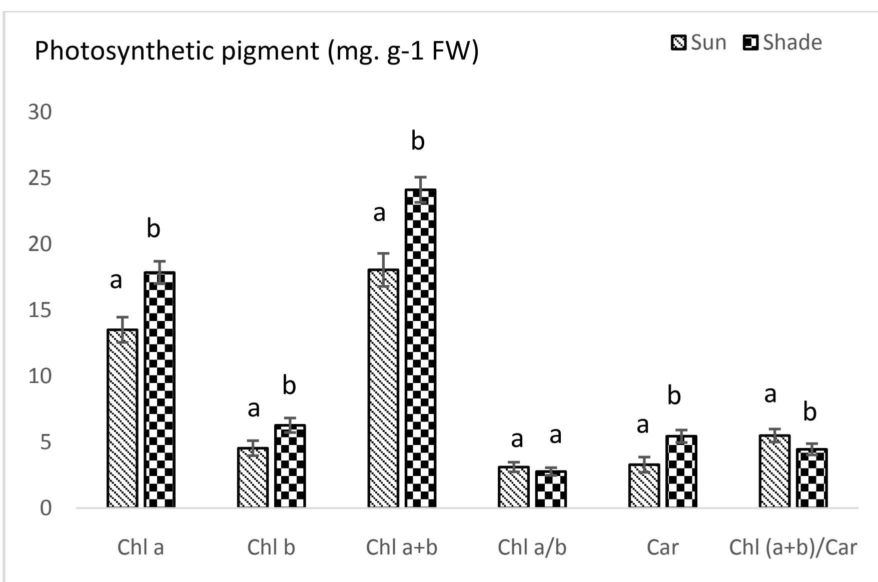
Maximum net photosynthetic rate, Amax; light compensation point, LCP; light saturation point, LSP; apparent quantum yield, AQY. Different small letters indicate significant differences between sun and shade leaves ($P < 0.05$).

The drier and warmer the air around a plant, the greater the driving force for water to move through a plant, thereby increasing the E (Sterling, 2005). The result of the experiment shows that sun leaves were having more stomatal density and less E, indicating that the plants use stomata to control water loss. This proves that more stomata minimize water loss from the plants. (Jifon & Syvertsen, 2003) explains that sun leaves have less E than shade leaves due to their thick cuticles.

3.2 Pigments

The total photosynthetic pigments of *V. amygdalina* are represented by figure 2. The result shows that shade leaves were having high content of Chl a, Chl b, and ratio of total Chl to car but lower ratio of Chl a to b, and lower car. This results can be explained by defining the meanings of the ratios. Chl a/b indicates how a plant adapt to light (H. K. Lichtenthaler & Buschmann, 2005). Even though the difference was not significant ($P > 0.05$), it agrees with the findings of (H. K. Lichtenthaler & Buschmann, 2005) who stated that Chl a/b is higher in sun leaves than in shade leaves because the former had lower Chl b, and can adapt to light more than the latter. Sun leaves on the other hand had higher carotenoids and lower chlorophyll contents. This may be due to the photo protective role of carotenoids.

The ratio of total Chl to car indicates the greenness of a leaf. The shade leafs studied were darker than the sun leafs studies. This is the reason why the shade leaves had higher ratio of total Chl to car. When comparing photosynthetic capacity per unit area of chlorophyll content, sun leaves had the highest capacity (K. Lichtenthaler et al., 2007).



Different small letters indicate significant differences between sun and shade leaves ($P < 0.05$)

Figure 2: The photosynthetic pigments of Vernonia amygdalina

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3.3 Stomatal density

The stomata of sun and shade leaves were studied, and the individual stomatal density was calculated. Sun leaves had the highest stomatal density (71 mm $^{-2}$) compared to shade leaves (50mm^{-2}) and the difference was statistically significant ($P < 0.05$). An additional study showed that there was a significantly positive correlation between stomatal density and the highest measured g_{sw} ($P < 0.05$). Stomatal density and highest E significantly reached negative correlation ($P < 0.01$) as represented by table 3. Difference in stomatal densities among species is mainly due to adaptation to environmental condition(Xu & Zhou, 2008). In this present study, our result shows that sun leaves were having higher stomatal density due to their adaptation to high light intensity while shade leaves were having lower stomatal density due to their adaptation to low light intensity.

Table 3: Pearson correlation coefficient among assimilation characteristics and stomatal density of the studied plants

	Sd (mm^{-2})	Anet (μmol CO2 m^{-2} s^{-1})	gsw (mol H2O m^{-2} s^{-1})	E (mol H2O m^{-2} s^{-1})
Sd	1			
A_{net}	.883*	1		
g_{sw}	.894*	.960**	1	
E	-.962**	.995**	.919**	1

Sd, stomatal density; A_{net}, net assimilation rate; g_{sw}, stomatal conductance to water vapor; E, transpiration rate.

**. Correlation is significant at the $P < 0.01$ level (2-tailed).

*. Correlation is significant at the $P < 0.05$ level (2-tailed).

Stomatal density affect the g_{sw}, A_{net} and E of plants. (Larcher, Hara-Nishimura, & Sternberg, 2015) reported that there is a statistically significant positive correlation among stomatal density and g_{sw}, A_{net}, and a negative correlation among stomatal density with specific leaf area of *Leymuschinensis*. In the same study, it was reported that as stomatal density increases, the water potential decreases. (Meng, Lei-xin, Wen-fu, Zheng-Jin, & Li-xia, 1999) reported that A_{net} is negatively correlated with stomatal density while the result of this study indicate that A_{net} is positively correlated ($P < 0.05$) with stomatal density. Our result shows that high number of stomata increases assimilation rate. In a study on the stomatal density and E of some plants, (Galmés, Flexas, Savé, & Medrano, 2007) reported that stomatal density do not correlate with E. the maximum g_{sw} of Mediterranean plants (Camargo & Marenco, 2011) correlates with the stomatal density.Red light decreases stomatal density of plants (Boccalandro et al., 2009). The studied sun leaves receives more blue light than the shade leaves and therefore the former have higher stomatal density than the latter. (Gitz & Baker, 2009) also reported that blue light increases the stomatal density of soy bean.In a previous study, light grown tomatoes were having higher stomatal densities than shade ones(Adedeji & Jewoola, 2008). They find out that the stomatal density of a matured leaf depend on the light history of the leaf as light affects stomatal development at the early stage of leaf development.

Water use efficiency of a plant decreases with increasing g_{sw}(Lambers et al., 2008). If there is water stress, plants reduce the stomatal opening in order to minimize water loss. If the plant is having low nitrogen, the stomata will open even if it will loss water (Lambers et al., 2008). The water saving of citrus leaves is higher when there is shading (Jifon & Syvertsen, 2003). The water saving of C3 plant falls between 2-11, while that of C4 plants is between 4-12 (Lambers et al., 2008). High stomatal density provides water saving in plants (Galmés et al., 2007).

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IV. Conclusion

The studied plantwas a light favouring species. There was significant variation in the gas exchange, photosynthetic pigments and stomatal densities of the studied plant depending on light availability. Sun leaves had higher gas exchange because they receive more light than shade leaves. Shade leaves had higher chlorophyll contents because they need to capture light. The higher stomatal density of sun leaves provides water saving to the plant.

References

[1] Adedeji, O., & Jewoola, O. a. (2008). Importance of Leaf Epidermal Characters in the Asteraceae Family. *Not. Bot. Hort. Agrobot. Cluj*, *36*(2), 7–16.

[2] Boccalandro, H. E., Rugnone, M. L., Moreno, J. E., Ploschuk, E. L., Serna, L., Yanovsky, M. J., & Casal, J. J. (2009). Phytochrome B Enhances Photosynthesis at the Expense of Water-Use Efficiency in Arabidopsis. *Plant Physiology*, *150*(2), 1083–1092.

[3] Camargo, M., & Marenco, R. (2011). Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. *Acta Amazonica*, *41*(2), 205–212.

[4] Edgar, S. A. (2014). *Analysis of phenolics from Centella asiatica and Vernonia amygdalina and their role as antibacterial and antioxidant compounds*.M.Sc thesis. Universiti Malaya, Mlaysia.

[5] Galmés, J., Flexas, J., Savé, R., & Medrano, H. (2007). Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: Responses to water stress and recovery. *Plant and Soil*, *290*(1–2), 139–155.

[6] Gitz, D. C., & Baker, J. T. (2009). Methods for Creating Stomatal Impressions Directly onto Archivable Slides. *Agronomy Journal*, *101*(1), 232.

[7] Jifon, J. L., & Syvertsen, J. P. (2003). Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree Physiology*, *23*(2), 119–127.

[8] Lambers, H., Chapin, F. S., & Pons, T. L. (2008). *Plant Physiological Ecology*. *Journal of Chemical Information and Modeling* (Vol. 53).

[9] Larcher, L., Hara-Nishimura, I., & Sternberg, L. (2015). Effects of stomatal density and leaf water content on the (18) O enrichment of leaf water. *The New Phytologist*, *206*(1), 141–151.

[10] Lichtenthaler, H. K., & Buschmann, C. (2005). Chlorophylls and Carotenoids: Measurement And Characterization by UV-VIS Spectroscopy. *Handbook of Food Analytical Chemistry*, *2–2*, 171–178.

[11] Lichtenthaler, K., Alexander, A., Marek, V., Kalina, J., & Urban, O. (2007). Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry*, *45*(8), 577–588.

[12] Liu, C., Guo, C., Wang, Y., & Ouyang, F. (2002). Effect of light irradiation on hairy root growth and artemisinin biosynthesis of Artemisia annua L. *Process Biochemistry*, *38*(4), 581–585.

[13] Ma, X., Song, L., Yu, W., Hu, Y., Liu, Y., Wu, J., & Ying, Y. (2015). Growth, physiological, and biochemical responses of Camptotheca acuminata seedlings to different light environments. *Frontiers in Plant Science*, *6*, 321.

[14] Marshal, B., & Biscoe, P. V. (1980). A Model for C 3 Leaves Describing the Dependence of Net Photosynthesis on Irradiance. *Journal of Experimental Botany*, *31*(1), 29–39.

[15] Meng, L., Lei-xin, L., Wen-fu, C., Zheng-Jin, X., & Li-xia, L. (1999). Effect of Water Stress on Stomatal Density,Length,Width and Net Photosynthetic Rate in Rice Leaves. journal of shenyang agricultural university, *5*, 1–5.

[16] Ogunlana, A. I. (2016). The antioxidant and phenolic profiles of five green. *Acta Scientiarum Polonorum Technol Aliment*, *15*(4), 391–397.

[17] Pengelly, J. J., Sirault, X. R., Tazoe, Y., Evans, J. R., Furbank, R. T., & von Caemmerer, S. (2010). Growth of the C4 dicot Flaveria bidentis: photosynthetic acclimation to low light through shifts in leaf anatomy and biochemistry. *Journal of Experimental Botany*, *61*(14), 4109–4122.

[18] Shirke, P. A., & Pathre, U. (2003). Diurnal and Seasonal Changes in Photosynthesis and Photosystem 2 Photochemical Efficiency in Prosopis juliflora Leaves Subjected to Natural Environmental stress. *Photosynthetica*, *41*(1), 83–89.

[19] State, A. (2016). Effect of debittering on phytochemicals and antioxidant composition of vernonia

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amygdalina and gongronema latifolium leaves. *International Journal of Applied and Natural Sciences*, *5*(2), 57–68.

[20] Sterling, T. M. (2005). *Transpiration - Water Movement through Plants*. New Mexico.

[21] Xu, Z., & Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, *59*(12), 3317–3325.

[22] Xue, W., Li, X.-Y., Zhu, J.-T., Lin, L.-S., & Wang, Y.-J. (2011). Effects of shading on leaf morphology and response characteristics of photosynthesis in Alhagi sparsifolia. *Chinese Journal of Plant Ecology*, *35*(1), 82–90.

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