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Research paper

Effect of Light on the Photosynthesis, Pigment Content and Stomatal Density of Sun and Shade Leaves of *Vernonia Amygdalina*

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Abstract

Light affects the growth and development of plants by influencing the physical appearance of one leaf as well as the appearance of the whole plant. Plant photosynthesis, stomata density, and pigment contents are all influenced by light The objective of this research is to determine the effect of light on the photosynthesis, pigment content and stomatal density of Sun and Shade Leaves of *Vernonia amygdalina*. Gas exchange was measured using Li-6400 and the data obtained was used to create a light response curve where parameters including light saturation point (LSP), light compensation point (LCP) and apparent quantum yield were estimated. Photosynthetic pigment were quantified spectrophotometrically. Moreover, the stomatal density was counted under light microscope, after making a nail polish impression of the leaf. The results discovered shows that as the light intensity increases, the gas exchange and stomatal density increases while the photosynthetic pigment of the studied plant decreases ($P<0.05$). In addition, LSP and LCP increases with increasing light intensity. Besides, statistically significant negative correlation ($P<0.05$) was achieved among stomatal density and transpiration rate thereby leading to a conclusion that sun leaves of *Vernonia amygdalina* contribute the highest assimilation rate to the plant than shade leaves. Yet, the higher stomatal density of sun leaves provides water saving to the plant.

Keywords: *gas exchange; light response characteristics; shade leaves; stomatal density; sun leaves; Vernonia amygdalina*

1. Introduction

Vernonia amygdalina is a shrub belonging to the family *Asteraceae*. The plant is native to tropical region especially Africa and it is otherwise called bitter leaf. It can be consumed as food or as a drug. 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, ethnopharmacology [1].

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 degree, and its surface characteristics. An important environmental condition affecting plants is light [2]. Light affects the growth and development of plants by influencing the physical appearance of a leaf as well as the appearance 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 plant changes the distribution of its biomass and its morphology, for a better approach to surviving [3].

Leaf photosynthesis, 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}) [4]. When the plant receives high light intensity, photoinhibition will occur because the excess photons would not be utilized in photosynthesis [5], creating an oxidative stress as a result of reactive oxygen species (ROS). For example, *Camptotheca acuminuita* grown at 75% irradiance have greater net photosynthesis rate than the plant grown under 100% irradiance [6]. Partially shaded plants usually have lower values of stomatal conductance to water vapor (g_{sw}) than unshaded plants. As a result of these differences, an objective to assess the changes in the photosynthesis, pigment content and stomatal density of sun and shade leaves of *Vernonia amygdalina* in response to varying light intensity is developed for this research.

2. Materials and Method

2.1 Plant material

Vernonia amygdalina having fully developed sun exposed and shaded leaves were sampled. Light intensity at the inner tree was about 100 µmol m^{-2} s^{-1} on sunny days, whilst that of the outer tree part was about 1800 µmol m^{-2} s^{-1}. Samples were harvested by cutting a small branch with the desired leaf and inserted in water. Furthermore, xylem embolisms was removed by cutting the branch under water [7].

2.2. Gas exchange measurements

Gas exchange was measured using LI-6400 (LICOR, Lincoln, Nebraska, USA) portable photosynthesis system. PPFD, Flow rate, leaf temperature, and CO2 were all controlled using the photosynthesis system. The system is an open gas mode. Youngest matured sun and shade leaves were randomly sampled. Light intensity used for generating the light response curve was in a decreasing order starting from 2000, 1500, 1250, 1000, 750, 500, 250, 100, 50, 25, to 0 µmol m^{-2} s^{-1}). Photo inhibition during measurements was avoided by acclimating each leaf before measurements. Moreover, the photosynthetic light response curve was plotted and fitted as bestowed by [8]. Beside, light response curve parameters were estimated using equation 1-3.

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

$$LCP = \frac{\theta\, x\, (R_d)^2 - R_d\, x\, A_{max}}{(R_d\, x\, \emptyset) - (\emptyset\, x\, A_{max})} \quad (2)$$

$$LSP = \frac{R_d + (0.9\ x\, A_{max})\, x\, A_{max} - \theta\, x\, (R_d + 0.9\ x\, A_{max})}{\emptyset(A_{max} - R_d} \quad (3)$$

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 [9]. 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.

Chl a (µg/ml) =13.36 $A_{664.1}$ – 5.19 $A_{648.6}$ (4)

Chl b (µg/ml) =27.43 $A_{648.6}$ – 8.12 A_664.1 (5)

Car (µg/ml) = ((1000A_{470} – 2.13a – 97.64b))/209 (6)

2.4. Determination of stomatal density

The stomatal density of the studied plant was evaluated by generating a nail polish leaf impression [10] on a slide. Observation was done using a light microscope at 40X objective lens. The area of field of view was divided by the average number of stomata in a field of view so as to obtain the stomatal density (mm-2).

2.5. Statistical analysis

All results were recorded after 3 experiments. The mean and standard deviation of each data was determined using SPSS statistics 20.0 (New York: IBM Corp.). Normality (Shapiro–Wilk normality test) testing was done due to the Pearson correlation coefficient among A_{net}, *E*, g_{sw} and stomatal density. Means were compared using T-test at a 95 % confidence level.

3. Results and Discussion

3.1. Photosynthesis

The photosynthetic light response curve fitted based on non-rectangular hyperbola model represent the effect of varying light intensity on photosynthesis of *Vernonia amygdalina* (figure 1). The curve defining the photosynthesis of the shade leaves is drastic while that of the sun leaves was abrupt. The light compensation point (LCP) and the light saturation point (LSP) indicates the gas exchange capacity of the plants. The results shows that the sun leaves had the highest gas exchange capacity per area as indicated in table 1. The studied sun and shade leaves were having an increasing assimilation rate as the light intensity increases from 0-250 µmol m^{-2} s^{-1}), nevertheless the assimilation continue to increase moderately reaching a maximum level, thereby forming a photosynthetic light response curve. Moreover, the photosynthesis parameters calculated were greater in sun leaves compared to shade leaves (Table 1). Gas exchange capacity of sun leaves were confirmed to vary significantly from that of shade leaves ($P < 0.05$) whilst the quantum yield did not differ between the leaves.

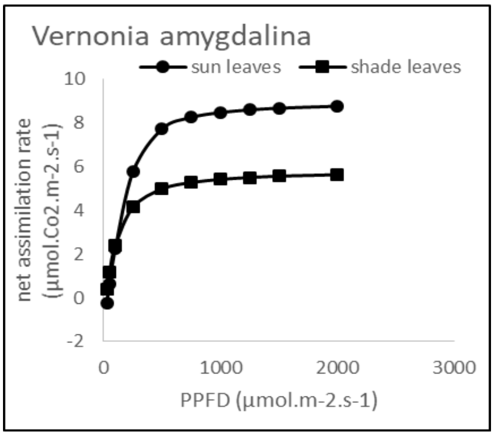

Fig. 1: The photosynthetic light response curve of *Vernonia amygdalina*

Sun exposed leaves usually differs from shaded leaves by having a higher LSP. Furthermore, the former have higher assimilation rate and abrupt curve curvature than latter. Higher number of chloroplast per area and higher number of stroma exposed thylakoid membranes of sun leaves leads to an increase in the assimilation rate of the leaves [11].
The result obtained indicated that the studied plant prefers higher light intensity for its maximum growth and photosynthesis because any decrease in the light intensity affects the assimilation rate, stomatal conductance and transpiration rate. Transpiration rate and stomatal conductance values differs between sun exposed and shaded leaves ($P < 0.05$), where the shaded leaves were having highest transpiration rate compared to the sun exposed 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)
S	10.10±0.46^a	31.47±1.31^a	1014.33±72.57^a	0.039±0.007^a
Sh	6.33±0.55^b	13.90±2.26^b	704.67±56.58^b	0.036±0.009^a

Sun exposed leaves, S; shaded leaves, Sh; Maximum net photosynthetic rate, A_{max}; light compensation point, LCP; light saturation point, LSP; apparent quantum yield, AQY. Different small letters indicate significant differences between sun and shade leaves ($P < 0.05$).

Moisture free and warm air increases the driving force for water to move through a plant this in return increases the transpiration rate of a plant [12]. The result of the experiment reveals the greater stomatal density of sun leaves and the less transpiration rate of the leaves, proving that the plants utilise their higher stomata density in controlling excessive loss of water. Another possible explanation of the lower transpiration rate of sun leaves can be due to the thick cuticles possessed by the leaves, which limit water loss [13].

3.2. Pigments

The chlorophyll and carotenoid contents of *V. amygdalina* are shown in Fig. 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 [9]. Even though the difference was not significant ($P > 0.05$), it agrees with the findings of [9] 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 [7].

3.3. Stomatal density

Sun leaves had the highest stomatal density (71 mm^{-2}) compared to shade leaves (50mm^{-2})and the difference was statistically significant ($P < 0.05$). Besides, a significant positive correlation was achieved between stomatal density and the highest measured stomatal conductance ($P < 0.05$). Stomatal density and highest transpiration rate measured achieved negative correlation ($P < 0.01$) as shown in Table 2. One important factor leading to variations in stomatal densities among species is differences in adaptation to environmental condition [10]. Adaptation of sun leaves to high light intensity makes them to have higher stomatal density whilst shade leaves adapt to shade conditions hence they achieved a low stomatal density.

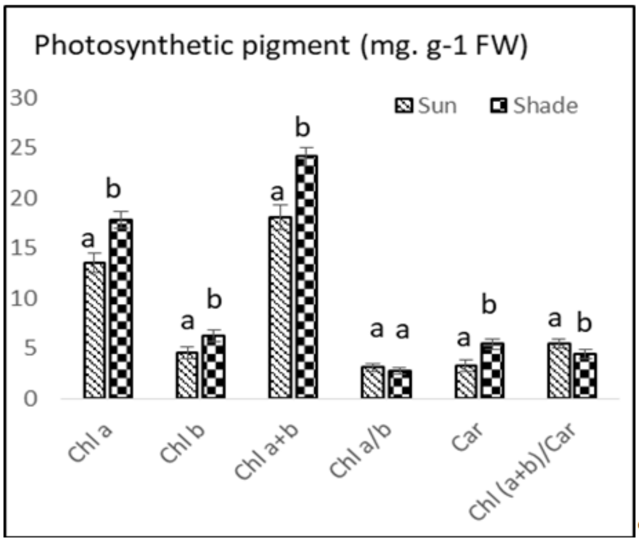

Fig. 2: The photosynthetic pigments of *Vernonia amygdalina*

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

Table 2: Pearson correlation coefficient among gas exchange characteristics and stomatal density of *Vernonia amygdalina*.

	Sd (mm^{-2})	A_{net} (µmol $CO2\ m^{-2}\ s^{-1}$)	g_{sw} (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).

The relationship between stomatal density and gas exchange characteristics is inevitable. Stomatal density was positively correlated with net assimilation rate and stomatal conductance, while a negative correlation was achieved among stomatal density and leaf area of *Leymus chinensis* [14]. Moreover, the authors reported that as stomatal density increases it lead to a decrease in the water potential of the studied plant. Meng et al. [15] 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, [16] reported that stomatal density do not correlate with *E*. the maximum g_{sw} of Mediterranean plants [17] correlates with the stomatal density. Red light decreases stomatal density of plants [18]. 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 [19] 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 [20]. 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} [11]. 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 [11]. The water saving of citrus leaves is higher when there is shading [13]. The water saving of C3 plant falls between 2-11, while that of C4 plants is between 4-12 [11]. High stomatal density provides water saving in plants [16].

4. Conclusion

The studied plant was a light favouring species. There was significant variation in the gas exchange, photosynthetic pigments and stomatal densities of the studied plant depeanding 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.

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