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Effect of light intensity on the gas exchange characteristics and total pigment content of *Psidium guajava*

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Abstract. The gas exchange characteristics and photosynthetic pigments of a plant can be affected by the amount of light received by the plant. The objective of this research is to investigate the effect of light intensity on the gas exchange characteristics and total pigment content TPC of sun and shade leaves of *Psidium guajava*. Sun and shade leaves were studied, and their photosynthetic light response characteristics including light saturation point (LSP), light compensation point (LCP) and apparent quantum yield were estimated. Carotenoids (Car) and chlorophyll (Chl) contents were quantified spectrophotometrically, while total anthocyanin content (TAC) was determined using the pH differential method. The result of this study shows that high light intensity influence gas exchange, carotenoid content and total anthocyanin content while low light intensity influence chlorophyll content especially chlorophyll b of the studied plant. As the light intensity increases, Amax, light saturation point and light compensation point increases. There are significant differences between the photosynthetic light response characteristics and total pigment content of sun and shade leaves ($P < 0.05$). The result leads to a conclusion that sun leaves of *Psidium guajava* contribute the greatest photosynthetic capacity to the plant than shade leaves. The high carotenoid and total anthocyanin contents of sun leaves provides photo protection to the plant.

1. Introduction

Psidium guajava from the family Myrtaceae is also known as common guava. It is a perennial, woody, shrub which can be consumed as food or as a drug. The plant is native to tropical America. *P. guajava* is now widespread throughout the tropics and subtropics [1]. It is a shade tolerant invasive species; that can survive for 40 years and popularly used for traditional medicine. It is a good source of vitamin C and pectin. The plant extracts is known to have antioxidant activity [2]. It also has anti diabetic properties [3]. Previous studies on *P. guajava* were mostly related to its medicinal effect, ethnopharmacology, and extraction of phenolic compounds.



Various environmental factors affects plant's gas exchange, structural and biochemical characteristics. One of the most significant factors is light. Light intensity can alter the shape, curling degree, and surface characteristics of a leaf [4]. 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 plant changes the distribution of its biomass and its morphology, in order to be able to utilize the amount of light they receive [5].

Leaf gas exchange and photosynthetic pigment 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 gas exchange (A_{net}) [6]. When the plant receives high light intensity, photoinhibition will occur because the excess photons will not be utilized in photosynthesis [7], leading to oxidative stress due to the large amount of reactive oxygen species (ROS). For example, *Torreya grandis* seedlings grown at 75% irradiance showed greater net photosynthesis rate than the plant grown under 90% irradiance [8]. Plants receiving lower light intensities usually have lower values of stomatal conductance to water vapour (g_{sw}) than plants receiving higher light intensities. Stomatal density is affected by altitude and light because it increases as the light intensity increases [9].

Anthocyanin are flavonoids produced by plants especially when they are under the influence of red and blue light [10]. They can protect the plant against ROS [11] and help in osmotic balance [12]. They are also produced in response to drought [13]. They therefore provide photo-protection and serve as antioxidants to plants [10]. They absorb and reduce concentration of blue light within a leaf, and convert the absorbed light to heat (light absorbed by anthocyanin is not use for photosynthesis).

The objective of this study is to investigate the effect of light intensity on gas exchange characteristics and total pigment content of *Psidium guajava*.

2. Materials and methods

2.1. Plant material

The study was performed by sampling fully developed sun and shade adapted leaves from the same plant. Shade leaves at the inner tree branches received less than $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ on sunny days, while sun leaves receives about $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). A branch with the desired leaf was cut from the tree and the cut end was immediately re-cut under water to remove xylem embolisms. The branch end remained in the water during the measurements [14]

2.2. Gas exchange measurement

The photosynthetic photon flux density was measured using LI-6400 (LICOR, Lincoln, Nebraska, USA) portable photosynthesis system quantum sensor. Flow rate of pump was constant, leaf temperature was 30°C while CO_2 was constant (ambient). 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 $0 \mu\text{mol m}^{-2} \text{s}^{-1}$). Photo inhibition was avoided during measurements by acclimating each leaf. This procedure was followed by light response curve which was later fitted according to [15]. Light response characteristics were estimated using Equation 1-3.

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

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

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

Where A_{net} : net photosynthetic rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); A_{max} : maximum photosynthetic rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); LCP: light condensation point ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); LSP: light saturation point at 90 % of A_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); ϕ : apparent quantum yield ($\text{mol CO}_2 \text{ mol photons}^{-1}$); R_d is dark (day) respiration rate (at no light; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); θ : curve convexity (no unit).

2.3. Determination of the total pigments contents

The total pigments concentration in sun and shade leaves of *Psidium guajava* was determined using UV visible spectrometer following [16]. The leaf used for gas exchange measurements was harvested, and transported to the laboratory, where it was weighed and homogenised in 95 % ethanol. Centrifugation was done at 2000g for 10 minutes at low temperature (4°C). The procedure was done in the dark so as to minimize pigment loss. Absorbance of supernatant was taken at 664.2 nm, 648.6 nm, and 470 nm respectively for Chl a, Chl b and carotenoids (Car) using a spectrophotometer. Total pigment content was calculated using Equation 4-6 and expressed in mg g^{-1} weight of fresh leaf.

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

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

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

2.4. Determination of the total anthocyanin contents

The total anthocyanin content present in sun and shade leaves of *P. guajava* was quantified using the pH differential method. 2.72g of sodium acetate was dissolved in 50ml distilled water and the pH was adjusted to 4.5. 0.37g of potassium acetate was dissolved in 200ml of distilled water and adjusted to pH 1.0. TAC was determined by performing different dilution of sample and buffer solution (maximum dilution was one part of sample in 4 parts of buffer solution). The absorbance was recorded within an hour of mixing the sample with the buffer, at 520nm and 700nm for both buffer solution dilutions. TFC was calculate using Equation 7, and expressed as cyanidine-3-glucoside equivalent ($\text{mg cy-3-glu L}^{-1}$).

$$\text{TAC} = \frac{(A \times \text{MW} \times \text{Df} \times 1000)}{(\epsilon \times 1)} \quad (7)$$

Where A is $(A_{520} - A_{700})_{\text{pH}1.0} - (A_{520} - A_{700})_{\text{pH}4.5}$, MW is molecular weight of cy-3-glu (449.2), Df is dilution factor, 1 is path length in cm, ϵ is molar extinction coefficient of cy-3-glu ($26,900 \text{ L.mol}^{-1} \text{ cm}^{-1}$).

2.5. Statistical analysis

All experiments were performed three times and the data were reported as the mean $\pm$ standard deviation. Statistical tests were performed using the IBM SPSS 20.0 (New York: IBM Corp.). Comparison of means using T-test (95% confidence) was done after testing the normality (Shapiro–Wilk normality test) of the data.

3. Results and discussion

3.1. Photosynthesis

The light response curve (Figure 1) indicates the effect of varying light intensity on gas exchange of *Psidium guajava*. The non-rectangular hyperbola model fitted light response curve had an acute curvature for shade leaves and a gradual one for sun leaves. The curve explains the gas exchange capacity of the plants which was indicated by the light compensation point (LCP) and the light saturation point (LSP). As shown in Table

1, sun leaves exhibit higher gas exchange capacity than shade leaves. Regardless of the sun exposure of the leaves, the A_{net} increases as the PPFD increased from 0-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A slow increase was achieved reaching a maximum level until the light response curve is completed. All light response characteristics were higher in sun leaves than in shade leaves ($P < 0.05$), except for apparent quantum yield (A_{qy}) which did not differ statistically

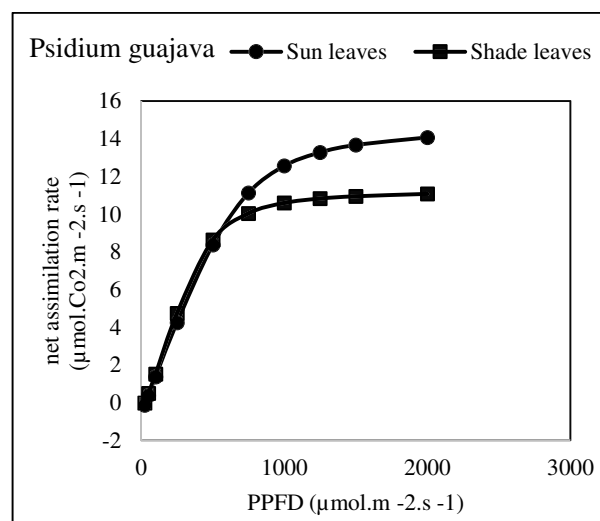


Figure 1. The photosynthetic light response curve of *P. guajava*

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. Gas exchange characteristics of *P. guajava* derived from light response curve

Species	A_{max} ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$)	LCP ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	LSP ($\mu\text{mol photons .m}^{-2}.\text{s}^{-1}$)	AQY ($\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$)
Shaded	15.47±1.25 a	31.41±1.71 a	1326.67±64.29 a	0.021±0.005 a
Sun	11.96±1.10 b	26.41±0.58 b	806.67±81.45 b	0.022±0.004 a

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 ($P < 0.05$).

The LCP indicates the capacity and ability to use lower light intensities. If a plant have lower LCP, it means the plant can utilize weak light more efficiently. Below the LCP, the light receive cannot compensate for the carbon loss during photo and dark respiration [17]. The shade leaves studied in this research have lower LCP than the sun leaves. This shows their greater ability to utilize lower light intensities. The LSP indicates the PPFD where the plant reach its maximum photosynthesis. If the LSP is low, it means the plants require lower PPFD to reach their maximum photosynthesis. The findings of this experiment shows that

shade leaves requires lower light intensities than sun leaves to reach their maximum photosynthesis. If a plant reach its LSP, the main limiting factor for photosynthesis will no longer be light, it will be the carboxylation rate [17]. Sun leaves differ from shade leaves by having a higher LSP. During this experiment, the leaf temperature was maintained so as to avoid any reduction in net assimilation rate because any increase or decrease in temperature affects the A_{net} [17]. Jifon and Syvertsen [18] reported that shade grapefruit and orange were having higher A_{net} due to lower leaf temperature.

P. guajava had a high photosynthesis rate which makes it an invasive species. Invasive species usually have higher A_{max} than native species. This makes them adapt to various environments and yield higher productivity [19]. A_{max} is higher in sun leaves because of their higher number of chloroplast per area, and their higher number of stroma exposed thylakoid membranes [17].

Chloroplast acclimation influence the curve curvature of the light response curve [20]. A_{qy} is similar in both sun and shade leaves. If shade leaves undergoes photo-inhibition or photo-destruction, then they will have lower A_{qy} than shade leaves [17]. In this study, the A_{qy} of sun leaves and shade leaves did not differ significantly.

The drier and warmer the air around a plant, the greater the driving force for water to move through a plant, thereby increasing the transpiration rate [21]. In the same study, it was explained that sun leaves have less transpiration rate than shade leaves due to their thick cuticles. Jifon and Syvertsen [18] recorded that 50 % shading do not have effect in the transpiration rate while our result shows that the higher the shading, the greater the transpiration rate. g_{sw} is affected by leaf temperature more than by humidity [18]. Difference in g_{sw} among species is mainly due to adaptation to environmental condition [22]. If a plant is having smaller guard cell size, their g_{sw} increases [23]. The result of our study indicates that shade leaves had lower g_{sw} than sun leaves. Lambers et al. [17] reviewed that water use efficiency of a plant decrease with increase in g_{sw} , transpiration rate increase with increase in g_{sw} , C_i increases with increase in g_{sw} , and A_{net} increases with increase in g_{sw} .

3.2. Total pigments contents

The total photosynthetic pigments of *P. guajava* 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 [16]. Lichtenthaler and Buschmann [16] stated that Chl a/b is higher in sun leaves than in shade leaves because the former can adapt to light more than the latter. Sun leaves on the other hand had higher Car and lower Chl contents. This may be due to the photo protective role of Car. The ratio of total Chl to Car indicates the greenness of a leaf. The shade leaves studied were darker than the sun leaves studied. This is the reason why the shade leaves had higher ratio of total Chl to Car. Sun leaves are reported to have the highest gas exchange capacity per unit area of chlorophyll content [14].

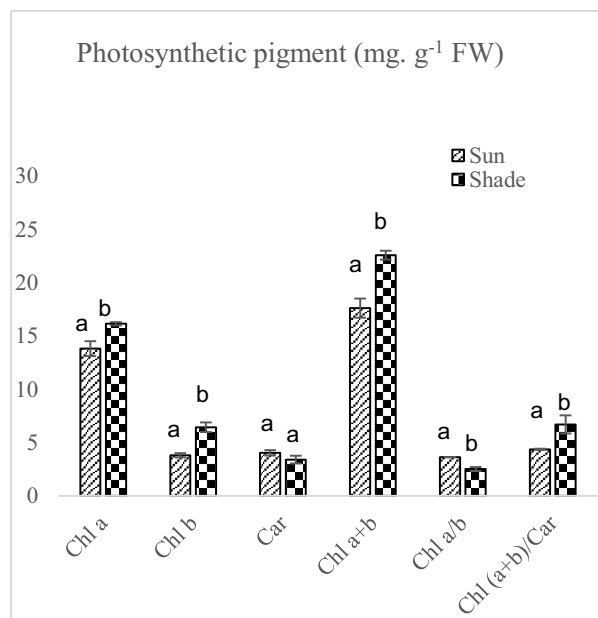


Figure 2. The photosynthetic CO₂ response curve of *P. guajava*.

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

3.3. Total anthocyanin content

The total anthocyanin content of sun leaves of *P. guajava* was 9.60 ± 0.73 mg/L while that of shade leaves was 7.11 ± 0.53 mg/L. Difference in TAC of sun and shade leaves was found to be statistically significant ($P < 0.05$). Anthocyanins are flavonoids (secondary metabolites) that have different roles in a plant including a photo protective role. Due to this, their concentration increases as the light intensity increases. Our result shows that *P. guajava* produces more anthocyanin under high light intensity. The mean TAC present in *P. guajava* leaves was recorded as 1.295% [24], 6.8 mg/100g in *P. guajava* nectar blend [25] and 0.24 mg/100g in *P. guajava* pulp [26].

Even though anthocyanin can be synthesised in almost all plant tissues, their synthesis can be influenced by prolonged exposure to high light intensity. This explains the higher TAC in sun exposed leaves, where the anthocyanin produced absorbs excess photosynthetic active radiation and reduce the light within the leaf. Anthocyanin content of *Zea mays* can absorb about 43% of PAR within a leaf [27].

4. Conclusion

Psidium guajava is a light favouring plant. There was significant variation in the gas exchange characteristics and TPC of the 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 for photosynthesis. Sun leaves contribute the greatest photosynthetic capacity to the plant than shade leaves. Also, the higher Car and anthocyanin contents of sun leaves provides photo protection to the plant.

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