

## EFFECT OF LIGHT INTENSITY ON THE PHOTOSYNTHESIS AND STOMATAL DENSITY OF SELECTED PLANT SPECIES OF GUNUNG LEDANG, JOHOR

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### ABSTRACT

Light intensity influences the photosynthesis and stomatal density in plants. In this study, it was hypothesized that if the transpiration rate of a plant is low, then the stomatal density might be high. The objective of this research is to investigate the effect of varying light intensity on the photosynthetic capacity and stomatal density of *Mikania micrantha*, *Tridax procumbens*, *Clidemia hirta* and *Centella asiatica* plant species of Gunung Ledang, Johor, Malaysia. Sun-exposed and shaded species were selected for the study. Gas exchange characteristics including stomatal conductance to water vapour ( $g_{sw}$ ), transpiration rate and intracellular CO<sub>2</sub> (Ci) were measured using LI-6400 portable photosynthesis system while net assimilation rate ( $A_{net}$ ), light saturation point (LSP), light compensation point (LCP), and apparent quantum yield ( $A_{qy}$ ) were calculated from the photosynthetic light response curve. Stomatal density was determined using nail polish impression method and examined using a stereo microscope. The result of this study shows that high light intensity influences the net assimilation rate and stomatal density of the studied plants. As the light intensity increases,  $A_{max}$ , LSP, LCP and stomatal density of the plants increases. There is a significant difference between the light response characteristics and stomatal density of the sun-exposed and shaded species ( $P < 0.05$ ). A statistically significant negative correlation ( $P < 0.05$ ) was achieved among stomatal density and transpiration rate. As stomatal density increases, the transpiration rate decreases. The result leads to a conclusion that *Mikania micrantha* can efficiently utilise the amount of light available more than *Tridax procumbens*, *Clidemia hirta* and *Centella asiatica* and grow abundantly as dominant species in an introduced environment.

**Key words:** Gas exchange characteristics, light response characteristics, sun-exposed plants, shaded plants, stomatal density

### INTRODUCTION

*Mikania micrantha* Kunth is a plant belonging to the Asteraceae family. It is known as Chinese creeper or American rope in English and Cheroma; Ulam tikas in Malaysia. This fast-growing vine is native to Central and South America. The plant can be found around the world, having a widespread distribution in Southeast Asia (GBIF Secretariat, 2018). *Tridax procumbens* is a plant belonging to the Asteraceae family. It is known as coat buttons in English. The plant is native to America, but presently it has a worldwide distribution. In Malaysia, it is known as Kanching baju. *Centella asiatica* is a plant belonging to the Apiaceae family. It is commonly known as Centella, Asiatic

pennywort or Gotu kola. It is native to the wetlands in Asia. It is used as a culinary vegetable and as a medicinal herb (CABI, 2019). *Clidemia hirta* is a plant belonging to the Melastomataceae family. It is known as Koster's curse. It is a perennial shrub which can be consumed as food or as a drug. The plant is native to the tropical region of America and now introduced across southern Asia, East Africa and Australia (Peters, 2001).

Light is an important environmental factor that affects plants photosynthesis and stomatal density. Plants differ in their response to light. For example, the photosynthesis of bayberry trees was inhibited at a high light intensity (Pan & Guo, 2016). *Camptotheca acuminata*, on the other hand, is having greater photosynthesis at a higher light intensity (Ma *et al.*, 2015). *Vernonia amygdalina* and *Mangifera indica* also have high photosynthesis

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under full sunlight (Idris *et al.*, 2018; Linatoc *et al.*, 2018). In a study of the effect of shading on the photosynthesis of *Anoetochilus roxburghii*, it was recorded that at 30% irradiance, *A. roxburghii* exhibits its highest net assimilation rate ( $A_{\text{net}}$ ) and stomatal conductance to water vapour ( $g_{\text{sw}}$ ) while the lowest  $A_{\text{net}}$  and  $g_{\text{sw}}$  was observed at 50% irradiance. As the light intensity increases, the  $A_{\text{net}}$ , transpiration rate and  $g_{\text{sw}}$  of *Piper betle* also increase (Shivashankara *et al.*, 2000). The  $A_{\text{net}}$  of *Tetrastigma hemsleyanum* was reported to be highest at 67% shade treatment (Dai *et al.*, 2009).

Stomatal density and index increases as light intensity increases (Volenikova & Ticha, 2001). Photoperiod also influences the stomatal density of plants (Casson & Gray, 2008). The lower concentration of  $\text{CO}_2$  and higher light intensity at higher altitude leads to an increase in stomatal density with altitude (Desilva *et al.*, 2014).

The transpiration rate of plants varies significantly. Some plants have higher transpiration rate in a shaded environment while others have higher transpiration rate at sun-exposed environment (Basahi *et al.*, 2014; Liu *et al.*, 2013; Pan & Guo, 2016; Tang *et al.*, 2015). Due to these variations, it was hypothesized in this current study that “if the transpiration rate of a plant is low, then the stomatal density might be high. The objective of this study is to evaluate how varying light intensity affects photosynthesis and stomatal density of the forest plants.

## MATERIALS AND METHODS

### Experimental site

The experiment was carried out in Gunung Ledang, Johor, Malaysia. The mountain is located at Tangkak area of Johor in Malaysia (02°22'27" North 102°36'28" East). The elevation is about 1,276 m. The average temperature and relative humidity of the forests was around 26°C and 92% in the rainy season and approximately 29°C and 83% in the dry season (Siddiki, 2015). The mean maximum Photosynthetic photon flux density (PPFD) was 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the sunny part of the forest and less than 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the deeply shaded part of the forest.

### Plant materials

Sun-exposed and shaded *Mikania micrantha*, *Tridax procumbens*, *Clidemia hirta* and *Centella asiatica* were selected for the study. The plants were sampled during day time hours at Gunung Ledang for studying the effect of varying light intensity on photosynthesis, and stomatal density. The study was performed from November 2017 to January 2018. The plants were selected because the research is

aimed at identifying the effect of light intensity on the photosynthesis and stomatal density of plants growing in their natural environment. Besides, the selected plants were found growing in sun-exposed and shaded regions of the forest. Plants from deeply shaded part of the forest (shaded-plants) and those directly under the sun (sun-exposed) were selected. Light intensity received at the sun-exposed regions is 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , while at the shaded region, it is below 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The light intensities were measured using LICOR quantum sensor.

### Determination of net assimilation rate ( $A_{\text{net}}$ )

PPFD was measured using LI-6400 (LICOR, Lincoln, Nebraska, USA) portable photosynthesis system quantum sensor. Net assimilation rate ( $A_{\text{net}}$ ) was determined using LI-6400. The youngest matured leaves of the plant (both sun-exposed and shaded plants) were randomly sampled. PPFD was variable (2000, 1500, 1250, 1000, 750, 500, 250, 100, 50, 25, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), leaf temperature was 30°C while  $\text{CO}_2$  was constant (ambient). Acclimation of each leaf (400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 1-3 minutes then 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 1-3 minutes) was done to avoid photoinhibition during measurements. Light response curve accompanied this procedure. Light response curve was fitted according to (Marshall & Biscoe, 1980) and the Light response curve characteristics were estimated using equation 1-3.

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

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

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

Where  $A_{\text{net}}$  is the net photosynthetic rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),

$A_{\text{max}}$  is the maximum photosynthetic rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),

LCP is the light compensation point ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),

LSP is the light saturation point calculated at 90% of  $A_{\text{max}}$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),

$\phi$  is the apparent quantum yield ( $\mu\text{mol CO}_2 \text{ mol photons}^{-1}$ ),

$R_d$  is daytime dark respiration rate (at no light;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), and

$q$  is curve convexity (dimensionless)

### Determination of stomatal density

This was achieved using the fingernail polish impression (Xu & Zhou, 2008), and observed using a stereomicroscope at 40× objective lens. The stomatal density ( $\text{mm}^{-2}$ ) was calculated by dividing

the area of the field of view by the average number of stomata in a field of view.

### Statistical analysis

All experiments were carried out in triplicates. Data were reported as the mean  $\pm$  standard deviation, and all tests were performed using the IBM SPSS statistics 20.0 statistical software program. The data were tested for normality (Shapiro–Wilk normality test) before the comparison of means. Student T-test was used to statistically analyse the data while Pearson correlation coefficient was used to evaluate the relationship between assimilation characteristics with stomatal density at 95% confidence level.

## RESULTS AND DISCUSSION

### Photosynthesis

The effect of varying light intensity on photosynthesis of some forest species 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 shaded plants is acute while that of the sun-exposed plants 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-exposed plants are

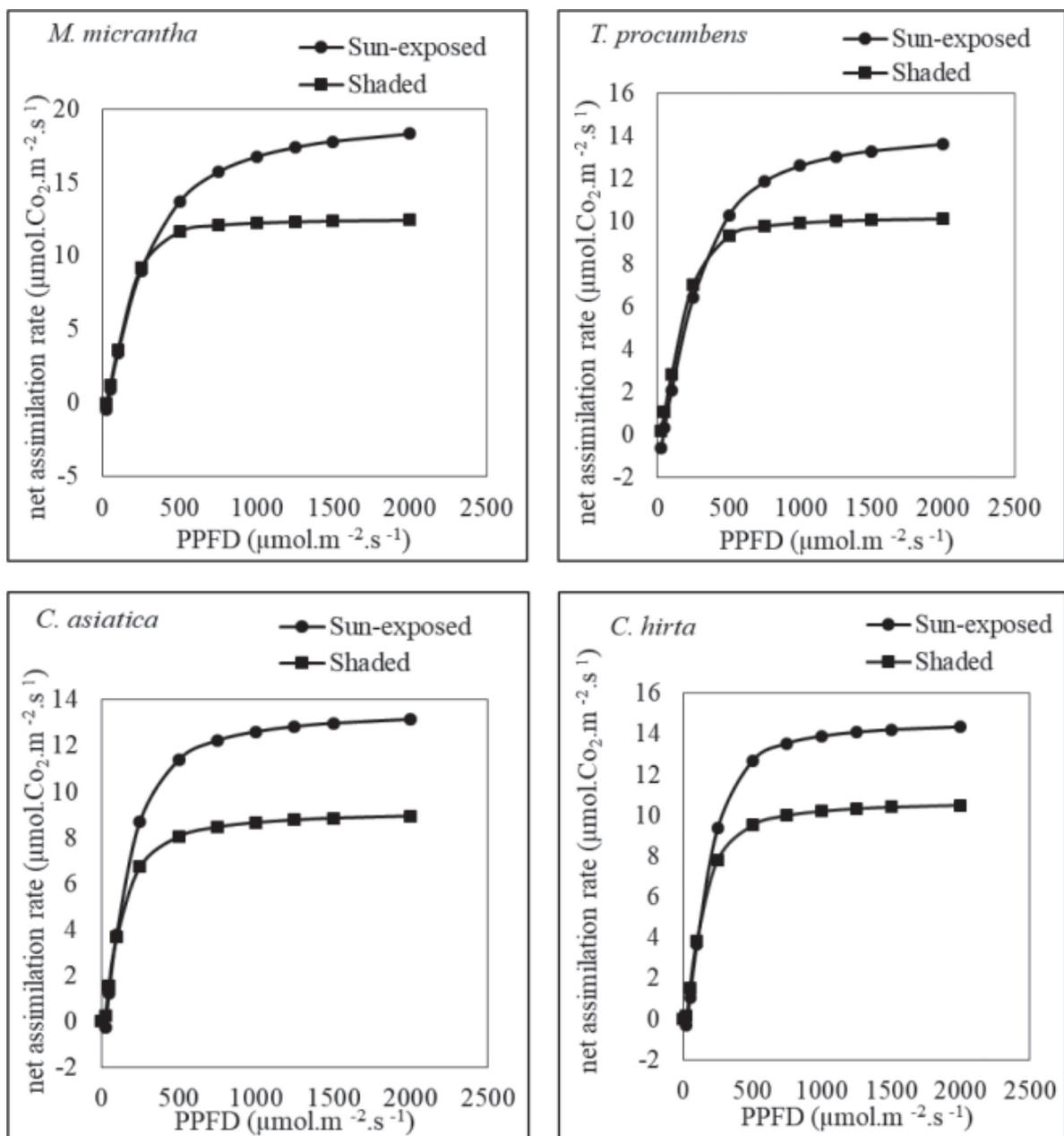


Fig. 1. The photosynthetic light response curve of the sun-exposed and shaded plant species.

**Table 1.** Light response characteristics of the studied plants

Species	LC	$A_{max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	LCP ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	LSP ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	$A_{qy}$ ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$ )
<i>M. micrantha</i>	SE	21.69 $\pm$ 1.52 <sup>A,a</sup>	33.20 $\pm$ 3.7 <sup>AB,a</sup>	1507.67 $\pm$ 112.81 <sup>A,a</sup>	0.059 $\pm$ 0.012 <sup>A,a</sup>
	SH	13.86 $\pm$ 0.89 <sup>A,b</sup>	25.20 $\pm$ 3.54 <sup>A,a</sup>	421.6667 $\pm$ 60.48 <sup>A,b</sup>	0.050 $\pm$ 0.018 <sup>A,a</sup>
<i>C. asiatica</i>	SE	15.42 $\pm$ 0.52 <sup>B,a</sup>	29.07 $\pm$ 4.71 <sup>B,a</sup>	998.33 $\pm$ 74.22 <sup>B,a</sup>	0.063 $\pm$ 0.015 <sup>A,a</sup>
	SH	10.39 $\pm$ 0.48 <sup>C,b</sup>	20.00 $\pm$ 3.61 <sup>A,a</sup>	490.67 $\pm$ 34.95 <sup>AB,b</sup>	0.060 $\pm$ 0.009 <sup>A,a</sup>
<i>T. procumbens</i>	SE	16.10 $\pm$ 0.20 <sup>B,a</sup>	41.70 $\pm$ 2.11 <sup>A,a</sup>	1245.33 $\pm$ 149.7 <sup>AB,a</sup>	0.039 $\pm$ 0.013 <sup>A,a</sup>
	SH	11.03 $\pm$ 0.31 <sup>BC,b</sup>	20.56 $\pm$ 6.07 <sup>A,b</sup>	480.01 $\pm$ 55.68 <sup>B,b</sup>	0.037 $\pm$ 0.009 <sup>A,a</sup>
<i>C. hirta</i>	SE	16.43 $\pm$ 0.45 <sup>A,a</sup>	30.90 $\pm$ 1.65 <sup>B,a</sup>	1166.67 $\pm$ 80.21 <sup>B,a</sup>	0.057 $\pm$ 0.017 <sup>A,a</sup>
	SH	12.07 $\pm$ 0.58 <sup>B,b</sup>	22.13 $\pm$ 2.01 <sup>A,b</sup>	563.33 $\pm$ 70.95 <sup>AB,b</sup>	0.055 $\pm$ 0.013 <sup>A,a</sup>

LC: light condition; SE: sun-exposed; SH: shaded;  $A_{max}$ : maximum net photosynthetic rate; LCP: light compensation point; LSP: light saturation point;  $A_{qy}$ : apparent quantum yield.

Different capital letters indicate significant differences among the plant species under the same light condition ( $P < 0.05$ ); Different small letters indicate significant differences among two light conditions of the same species ( $P < 0.05$ ).

having greater photosynthesis capacity per area as shown in Table 1. In both sun-exposed and shaded plants,  $A_{net}$  increases as the PPFD increased from 0-250  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  and then the assimilation increases slowly to a maximum level forming a light response curve. All photosynthesis parameters were higher in sun-exposed species compared to shaded species (Table 1).  $A_{max}$  and  $A_{net}$  of sun-exposed species of all plants varied significantly ( $P < 0.05$ ) from  $A_{max}$  and  $A_{net}$  of shaded species. LCP and LSP of sun-exposed species varied significantly from LCP and LSP of shaded species ( $P < 0.05$ ). Both plants'  $A_{qy}$  did not differ significantly between the sun-exposed and shaded species.

LCP indicate the gas exchange capacity of the plants. The results obtained indicate that shaded plants were having lower LCP compared to sun-exposed plants ( $P < 0.05$ ). Among the selected plants, sun-exposed *T. procumbens* was having the highest LCP (41.70  $\pm$  2.11  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), while shaded *C. asiatica* was having the lowest LCP (20.00  $\pm$  3.61  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Plants with lower LCP can use light more efficiently than the plants with higher LCP because LCP indicates the plant's ability to use light efficiently (Lambers *et al.*, 2008). If the LCP of a plant is low, it means a plant can be able to photosynthesize at a lower light intensity more than plants with higher LCP. Nevertheless, below the LCP, a plant cannot compensate for the carbon loss during respiration. In other words, the LCP is the point where plants photosynthesis matches the rate of cellular respiration (Lambers *et al.*, 2008).

Most sun-exposed leaves have higher LCP, even though there are exceptions, for example; shaded leaves of *Aeschynanthus longicaulus* (Li *et al.*, 2014), *Tetrastigma hemsleyanum* (Dai *et al.*, 2009), and *Lindera melissifolia* (Aleric & Kirkman, 2005) had higher LCP compared to the sun-exposed species. This may be due to light unification and variation in response to light. In another study, the LCP of shaded *C. hirta* was reported to be 6  $\mu\text{mol}$

$\text{photons m}^{-2} \text{ s}^{-1}$  while that of sun-exposed *C. hirta* was recorded to be between 9–13  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  depending on the cultivar type (DeWalt *et al.*, 2004). The LCP of *M. micrantha* recorded in this current study is 25.20  $\pm$  3.54 - 33.20  $\pm$  3.7  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , while that obtained by Shen *et al.* (2007) ranges between 22–28  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . This indicates that variations in response to light exist within and between species.

LSP also indicates the gas exchange capacity of the plants. The LSP of the selected plants is higher in sun-exposed compared to shaded leaves ( $P < 0.05$ ). Among the selected plants, sun-exposed *M. micrantha* were having the highest LSP (1507.67  $\pm$  112.81  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), while shaded *M. micrantha* recorded the lowest LSP (421.6667  $\pm$  60.48  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). From the results obtained, it can be interpreted that sun-exposed *M. micrantha* requires maximum PPFD to reach its maximum photosynthesis. This is because LSP is the PPFD where a plant reaches its maximum photosynthesis. If the LSP is low, it means a plant requires lower PPFD to reach its maximum photosynthesis. Above the LSP, light is no longer the limiting factor for photosynthesis, but rather, the carboxylation rate determines the rate of photosynthesis (Lambers *et al.*, 2008). Sun-exposed plants tend to have higher LSP compared to semi-shaded or shaded species (DeWalt *et al.*, 2004; Lambers *et al.*, 2008; Li *et al.*, 2016; Liu *et al.*, 2013; Qin *et al.*, 2012; Xue *et al.*, 2011). Sun-exposed species differ from shaded species by having a higher LSP. In another study, LSP of *M. micrantha* was 985  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Shen *et al.*, 2007). Compared to the LSP of *C. hirta* obtained in this study and that obtained by DeWalt *et al.* (2004), the LSP obtained in the former are higher than that of the latter, thus indicating that high variation in response to light exists within species. Ghale (2013) reported that the LSP of sun-exposed *M. micrantha* was above 800  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  while that of shaded species was 500  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ .

As the light intensity increases, the  $A_{\text{net}}$  also increases. The  $A_{\text{net}}$  of sun-exposed *M. micrantha* was twice higher than that of shaded *M. micrantha* as reported by Ghale (2013).  $A_{\text{max}}$  is the maximum net assimilation rate that occurs at the LSP. In other words, it is the net assimilation ( $A_{\text{net}}$ ) plus the dark respiration. As the light intensity increases, the  $A_{\text{net}}$  increases. This, therefore, increases the  $A_{\text{max}}$  of the plant. The studied sun-exposed species were having higher  $A_{\text{max}}$  compared to the semi-shaded and shaded species ( $P < 0.05$ ). The highest  $A_{\text{max}}$  was obtained in sun-exposed *M. micrantha* ( $21.690 \pm 1.52 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) while the lowest was recorded for shaded *C. asiatica* ( $10.39 \pm 0.48 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Plants with high  $A_{\text{max}}$  may contain a higher number of chloroplast per area. Also, a higher number of stroma-exposed thylakoid membrane may be present in the sun-exposed species (Lambers *et al.*, 2008). Plants can differ in their  $A_{\text{max}}$  depending on the level of light intensity received. For example, *Chromera velia* (Belgio *et al.*, 2017) had higher  $A_{\text{max}}$  when grown under high light while *Changium smyrnioides* (Wang *et al.*, 2017) had higher  $A_{\text{max}}$  when grown under a moderate amount of shading. In another study, the highest value of  $A_{\text{max}}$  of *M. micrantha* was also recorded in sun-exposed species (Zhang & Wen, 2009). Values obtained in this study were similar to the values of net assimilation of *M. micrantha* obtained in another study (Ghale, 2013). Moreover,  $A_{\text{max}}$  of *M. micrantha* was found to be above  $18 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Zhang & Wen, 2009; Shen *et al.*, 2007). In another study,  $A_{\text{max}}$  of *C. hirta* was found to range between  $11\text{--}15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  for shaded and sun-exposed species respectively, while that obtained in this study ranges between  $7.49 \pm 0.94$  to  $16.39 \pm 0.56 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  respectively. A possible explanation for differences in  $A_{\text{max}}$  of the species may be due to the existence of wide variation in response to light in plant species. Invasive species usually have higher  $A_{\text{max}}$  than native species. This makes them have higher productivity (Heberling & Fridley, 2013). The studied plants are invasive species with high  $A_{\text{max}}$ . Sun-exposed species have higher  $A_{\text{max}}$  and lower curve curvature than shaded species. The studied plants can grow best at a high light intensity. This agrees with the findings of Sterling (2005) who concluded that *M. micrantha* has high LSP and  $A_{\text{net}}$  at a higher light intensity and can grow best at a high light intensity.

The slope of the light response curve represents the apparent quantum yield ( $A_{\text{qy}}$ ). The slope of the curve can be seen in Figure 1. The differences in the mean of  $A_{\text{qy}}$  of sun-exposed and shaded plants are not significant ( $P > 0.05$ ). The results obtained for sun-exposed and shaded species are not significant because  $A_{\text{qy}}$  is similar in both sun-exposed and

shaded leaves (Lambers *et al.*, 2008).  $A_{\text{qy}}$  is a representation of a plants efficiency to convert light into fixed carbon. In some situations, for instance, when the shaded plant undergoes photoinhibition, it tends to have lower  $A_{\text{qy}}$  compared to sun-exposed species (Lambers *et al.*, 2008). In *Artemisia annua*,  $A_{\text{qy}}$  did not differ among species grown at 30%, 50% and 100% sunlight (Wang *et al.*, 2007). In *Alhagi sparsifolia*,  $A_{\text{qy}}$  did not differ among species grown under full sunlight and those grown under shade (Xue *et al.*, 2011). Likewise in *Arabidopsis thaliana* (Violet-Chabrand *et al.*, 2017) and *Typha latifolia* (Jespersen *et al.*, 2017),  $A_{\text{qy}}$  did not differ among sun-exposed and shaded species. The  $A_{\text{qy}}$  recorded for *M. micrantha* in this current study is  $0.050 \pm 0.018 - 0.059 \pm 0.012 \text{ mol CO}_2 \text{ mol}^{-1} \text{ photons}$ , which is similar to the  $A_{\text{qy}}$  obtained in another study ( $0.05 - 0.06 \text{ mol CO}_2 \text{ mol}^{-1} \text{ photons}$ ) (Shen *et al.*, 2007). In *C. hirta*, it was reported that the  $A_{\text{qy}}$  of shaded and sun-exposed species was  $0.083$  and  $0.079 \text{ mol CO}_2 \text{ mol}^{-1} \text{ photons}$  for (DeWalt *et al.*, 2004), which are higher than that obtained in this present study. Therefore, high variation in response to light exists within and between plant species.

A decrease in light intensity leads to a decrease in  $A_{\text{net}}$ , transpiration rate and  $g_{\text{sw}}$  (Table 2). Transpiration rate and  $g_{\text{sw}}$  values varied significantly between sun-exposed and shaded species. Shaded species were having greater transpiration rate than sun-exposed species ( $P < 0.05$ ).  $A_{\text{net}}$  of sun-exposed species was significantly higher than that of shaded species ( $P < 0.05$ ). In this study, it was hypothesized that if the transpiration rate of a plant is low, then the stomatal density might be high. The result of the experiment shows that sun-exposed species were having more stomatal density and less transpiration rate, indicating that the plants use stomata to control water loss. The findings lead to accepting the tested hypothesis for the study and shows that more stomata minimize water loss from the plants. Jifon and Syvertsen (2003) explains that sun leaves have less transpiration rate than shaded leaves due to their thick cuticles while Sterling (2005) reported that 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. Gale, (2004) recorded that 50% shading does not have any effect on the transpiration rate while our result shows that the higher the shading, the greater the transpiration rate. Jifon and Syvertsen (2003) reported that higher altitudes increase transpiration rate compared to lower altitudes.

### Stomatal density

The stomata of sun-exposed and shaded species were studied, and the individual stomatal density was calculated. The plants have mainly anomocytic

**Table 2.** Assimilation characteristics and stomatal density of the studied plants

	LC	$A_{net}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	SD ( $\text{mm}^{-2}$ )	$g_{sw}$ ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	E ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )
<i>M. micrantha</i>	SE	18.33±1.34 <sup>A,a</sup>	184±4.00 <sup>A,a</sup>	0.46±0.06 <sup>AB,a</sup>	3.11±0.12 <sup>A,a</sup>
	SH	11.9±1.00 <sup>A,b</sup>	148±2.00 <sup>A,b</sup>	0.70±0.05 <sup>A,b</sup>	3.77±0.33 <sup>AB,b</sup>
<i>C. asiatica</i>	SE	12.77±1.00 <sup>B,a</sup>	104±5.00 <sup>B,a</sup>	0.47±0.08 <sup>AB,a</sup>	2.90±0.10 <sup>B,a</sup>
	SH	8.63±0.42 <sup>B,b</sup>	93±3.51 <sup>B,b</sup>	0.600±0.100 <sup>AB,a</sup>	3.97±0.95 <sup>B,a</sup>
<i>T. procumbens</i>	SE	13.5±0.56 <sup>B,a</sup>	83±3.00 <sup>C,a</sup>	0.31±0.02 <sup>A,a</sup>	1.9±0.11 <sup>B,a</sup>
	SH	9.83±0.78 <sup>B,b</sup>	52±3.00 <sup>C,b</sup>	0.42±0.03 <sup>B,b</sup>	3.24±0.12 <sup>AB,b</sup>
<i>C. hirta</i>	SE	13.67±1.53 <sup>B,a</sup>	265±3.00 <sup>D,a</sup>	0.62±0.11 <sup>B,a</sup>	2.27±0.25 <sup>AB,a</sup>
	SH	10.17±0.64 <sup>AB,b</sup>	184±4.00 <sup>D,b</sup>	0.697±0.095 <sup>A,a</sup>	2.47±0.503 <sup>A,a</sup>

LC: light condition; SE: sun-exposed; SH: shaded;  $A_{net}$ : net assimilation rate; SD: stomatal density;  $g_{sw}$ : stomatal conductance to water vapour; E: transpiration rate.

Different capital letters indicate significant differences among the plant species under the same light condition ( $P < 0.05$ ); Different small letters indicate significant differences among two light conditions of the same species ( $P < 0.05$ ).

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

	Stomatal density			
	<i>M. micrantha</i>	<i>C. asiatica</i>	<i>T. procumbens</i>	<i>C. hirta</i>
$A_{net}$	.930**	0.804	.939*	0.872*
$g_{sw}$	-.937**	-0.316	-.923**	-.437*
E	-.855*	-0.661	-.968**	-.264**

$A_{net}$ : net assimilation rate;  $g_{sw}$ : stomatal conductance to water vapor; E: transpiration rate.

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

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

and anisocytic stomata. In all studied plants, the stomatal density of sun-exposed species is higher than that of shaded species ( $P < 0.05$ ) (Table 2). The stomatal densities of the plant species under the same light condition are statistically significant in most of the comparisons ( $P < 0.05$ ). The measured stomatal conductance ranges from 0.1 – 0.7  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  among all studied plants. The mean maximum  $g_{sw}$  achieved differs between sun-exposed and shaded species ( $P < 0.05$ ).

An additional study showed that there was a significantly positive correlation between stomatal density and measured  $g_{sw}$  ( $P < 0.01$ ). Stomatal density and transpiration rate significantly reached negative correlation ( $P < 0.05$ ) as represented in Table 3. The difference in stomatal densities among species is mainly due to the adaptation to environmental condition (Xu & Zhou, 2008). In this present study, our result shows that sun-exposed species were having higher stomatal density due to their adaptation to high light intensity while shaded species were having lower stomatal density due to their adaptation to lower light intensity.

Stomatal density affects the  $g_{sw}$ ,  $A_{net}$  and transpiration rate of plants. Larcher *et al.* (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 a specific leaf area of *Leymus chinensis*. As the stomatal density of *Leymus chinensis* increases, the water potential decreases (Xu & Zhou 2008). Meng *et al.* (1999) reported that  $A_{net}$  is negatively correlated with stomatal density while the result of this study indicate that  $A_{net}$  is positively correlated with stomatal density. Our result shows that a high number of stomata increases assimilation rate. In a study on the stomatal density and transpiration rate of some plants, Galmés *et al.* (2007) reported that stomatal density does not correlate with transpiration rate while in another study, it was reported that the maximum  $g_{sw}$  of Mediterranean plants (Camargo & Marengo, 2011) correlates with the stomatal density.

Red light decreases the stomatal density of plants (Boccalandro *et al.*, 2009). The sun-exposed species receive more blue light than the shaded species and therefore the former have higher stomatal density than the latter. Gitz and Baker (2009) also reported that blue light increases the stomatal density of soybean. In a previous study, sun-exposed coffee plants were having higher stomatal densities than shaded ones (Pompelli *et al.*, 2010). They find out that light affects stomatal development at the early stage of leaf development. This means that the stomatal density of a matured leaf depends on the light history of the leaf.

## CONCLUSION

The studied plants were light favouring species. There was significant variation in the  $A_{net}$  and stomatal densities of the studied plants depending on light availability. The plants grow best at the high light condition. Even though *C. asiatica*, *T. procumbens* and *C. hirta* are regarded as invasive species, they cannot utilise light more efficiently compared to *M. micrantha*. The higher stomatal density and effective utilisation of light make *M. micrantha* grow abundantly as a dominant invasive species in an introduced environment. In conclusion, the studied plants were invasive species that grow fast and accumulate biomass due to their efficient utilisation of light. The plants can be used for bioremediation, rehabilitation and soil improvement applications due to their high photosynthetic rates.

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