

# Photosynthetic capacity, photochemical efficiency and chlorophyll content of three varieties of *Labisia pumila* Benth. exposed to open field and greenhouse growing conditions

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**Abstract** Three varieties (*Alata*, *Pumila* and *Lanceolata*) of Malaysian indigenous medicinal herb *Labisia pumila* Benth. grown in greenhouse and open field were tested to evaluate and compare their photosynthetic and maximal quantum efficiency of photosystem II ( $F_v/F_m$ ) reactions. Every variety grown in greenhouse demonstrated higher light-saturated photosynthetic capacity than in the open field. The diurnal net photosynthesis ( $A$ ) curve in the open field also displayed dual peaks with lower daily average  $A$  compared to the greenhouse. Varieties *Alata* and *Pumila* were found to acclimatize better under both growing conditions. The diurnal patterns of  $F_v/F_m$  indicated that plants grown under greenhouse encountered less photoinhibition than in open field condition. A decrease in chlorophyll (chl)  $a/b$  ratio in leaves of greenhouse plants with significant increase in chl  $b$  was observed. This study indicates that var. *Alata* and var. *Pumila* have the capacity to acclimatize to greenhouse growth condition.

**Keywords** Chlorophyll fluorescence · *Kacip Fatimah* · Net photosynthesis · Photoinhibition · Photosynthetic photon flux density · Photosystem II

## Introduction

*Labisia pumila* Benth. is a sub-herbaceous plant with creeping stems from the family Myrsinaceae that is found widespread in Malaysian forest. It is an indigenous, understory medicinal herb of Malaysia, popularly known as *Kacip Fatimah*, and sometimes also referred locally as *Selusah Fatimah*, *Rumput Siti Fatimah*, *Akar Fatimah*, *Tadah Matahari*, *Bunga Belangkas Hutan* and *Pokok Pinggang* (Jamia et al. 2003). It has customarily been used by Malay women to induce and facilitate childbirth as well as a post-partum medicine (Burkill 1966). Stone (1988) categorized the three varieties of this herb in Malaysia as *L. pumila* var *Alata*, *L. pumila* var *Pumila* and *L. pumila* var *Lanceolata*. Each of the beneficial varieties has different usage. But the most universally utilized varieties by traditional healers are *L. pumila* var *Alata* and var *Pumila*. Other uses of this herb are for dysentery, dysmenorrhea, flatulence and gonorrhoea treatments (Rozihawati et al. 2003). Due to the recent discovery of the estrogenic activities of this herb (Ezumi et al. 2006), the demand for this herb in the recent years has increased tremendously in the herbal and commercial industry (Jaafar et al. 2008).

Presently, *L. pumila* has been abundantly collected from the rainforest to suffice industry requirement. This system of harvest in Malaysia results in an unreliable supply and also inconsistent quality of this herb (Jaafar et al. 2008). With increasing demand for this herb over the years, the authors were concerned that it might endanger the species existence in nature and cause a loss of biodiversity to Malaysia. The effort to domesticate this herb commercially has not been too encouraging, due to the difficulty in raising the species away from its natural habitat. The plant is not only slow growing, but also, as highlighted by Jaafar et al. (2009), the species is very

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sensitive to changes in microclimate especially temperature, light and relative humidity. Therefore, the authors suggest that growing *L. pumila* under greenhouse could be one of the best ways to produce raw materials of this herb commercially, as the microenvironment of the growing plants can be controlled and manipulated. The productivity of *L. pumila* is a function of the production of assimilates by photosynthesis and translocation of assimilates to plant sinks (Jaafar et al. 2010). The alterations of photosynthesis rate ( $A$ ) can also influence the plant growth and development processes (Lei et al. 1996). Under greenhouse condition, light is one of the important microclimate factors that influence plant productivity apart from temperature, relative humidity and  $\text{CO}_2$  concentrations. Despite the necessity of light for autotrophic organisms, no plant is capable of using 100% of solar irradiation for photosynthesis (Demmig et al. 1997). During plant acclimation to specific light environment, chlorophyll concentration, maximal quantum yield for photosystem II ( $F_v/F_m$ ), chloroplast density, expression of antioxidant enzymes and Calvin cycle intermediate have been shown to fluctuate during this process (Demmig et al. 1997; Hernandez et al. 2004; Yoshimura et al. 2000). Nevertheless, little information is available on the photosynthetic acclimation responses of *L. pumila* either in the open field or greenhouse condition, which may be the basis for variety selection for commercial cultivation.

Plants grown in greenhouse and open field conditions vary their growth periods and growth responses very significantly, partly due to differences in the microclimates. In the open field, most plants receive more sunlight than they can actually use for photosynthesis, as a result photosynthesis produces more NADPH and ATP than required. This accumulation of redox and energy will decrease the plastoquinone pool and inhibit the water splitting complex leading to PSII inactivation, the so-called photoinhibition (Karpinski et al. 1997). Under these circumstances, plants have to exhaust the excess light energy that had been absorbed. This will depend on a protective non-photochemical mechanism that quenches singlet-excited chlorophyll and harmlessly scours excess excitation energy as heat. The non-photochemical quenching (NPQ) process occurs in all photosynthetic eukaryotes and helps to control and shield photosynthesis in the environment, in which light energy absorption exceeds the capacity for light utilization (Patricia et al. 2001).

The main objective of this research was to determine the differences in photosynthetic responses of three varieties of *L. pumila* Benth. to different growing conditions (photosynthetic photon flux density, temperature,  $\text{CO}_2$  concentration) in the open field and greenhouse. We would also like to establish whether these varieties showed the ability to acclimatize under greenhouse conditions, which could

ultimately be the basis for variety selection raised under greenhouses for commercial use.

## Materials and methods

The site is situated at Field 2, University Agriculture Park, Universiti Putra Malaysia (longitude  $101^\circ 44' \text{N}$  and latitude  $2^\circ 58' \text{S}$ , 68 m above sea level) with a mean atmospheric pressure of 1.013 kPa. Three varieties of *L. pumila* used in this study, namely var *Alata*, *Pumila* and *Lanceolata*, were grown in a nursery plot in a greenhouse complex of the faculty of agriculture and measured during November of 2009. The seedlings were planted in soilless medium containing coco-peat, burnt paddy husk and well composted chicken manure in 5:5:1 (v/v) ratio in 25-cm diameter polyethylene bags. Day and night temperatures in the greenhouse were maintained at  $27\text{--}30^\circ\text{C}$  and  $18\text{--}21^\circ\text{C}$ , respectively, and relative humidity from 50 to 60%. Organic fertilizer was applied every 2 weeks using well-composted chicken manure at 20 g/plant. All the seedlings were irrigated using overhead mist irrigation given four times a day or when necessary. Each irrigation session lasted for 7 min.

A two-factor experiment was organized in a completely randomized design (CRD) with three varieties of *L. pumila*: var *alata* (Alata), var *pumila* (Pumila) and var *lanceolata* (Lanceolata) and two growing conditions (open field and greenhouse) replicated three times, and each treatment consisted of five plants. Standard error means (S.E.M.) was calculated and differences in mean values for each treatment were tested at  $p \leq 0.05$  according to Duncan multiple range test (DNMRT).

Total chlorophyll content was measured using a method from Hardwich and Baker (1973) based on fresh weight basis. Prior to each destructive harvest, seedling was analyzed for the leaf relative chlorophyll reading (SPAD meter 502, Minolta Inc, USA). The leaves of *Labisia pumila* with different greenness (yellow, light green and dark green) were selected for analysis and total leaf chlorophyll content was analyzed. For each type of leaf, the relative SPAD value was recorded (5 points/leaf) and sampled for chlorophyll content determination. Leaf disk 3 mm in diameter were obtained from leaf sample using a hole puncher. For each seedling, the measurements were conducted on the young fully expanded leaves; generally, on the second or third leaf from the tip of the stem was used. The leaf disks were immediately immersed in 20 ml of acetone in an aluminum foil-covered glass bottle for approximately 24 h at  $0^\circ\text{C}$  until all the green color had bleached out. Finally, 3.5 ml of the solution was measured using a spectrophotometer (UV-3101P, Labomed Inc, USA) at absorbances of 664 and 647 nm. Using the data obtained, the least squares

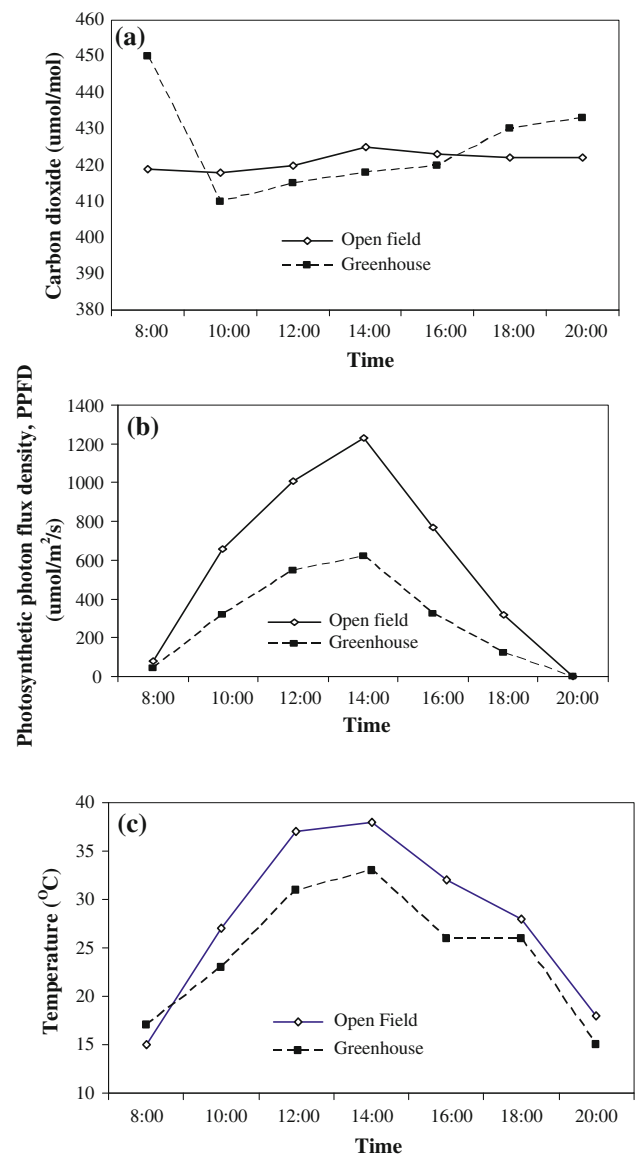
regression was established to develop a predictive relation between pigment concentrations (mg/g fresh weight) attained from the destructive chlorophyll method and relative chlorophyll content from the SPAD meter readings.

Leaf gas exchange measurements were taken using closed system, infrared gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA: LICOR Inc, Lincoln, NE, USA) set with optimal growth conditions by placing the cuvette head over fully expanded leaf supported by a tripod stand. Prior to the measurement, the maximum photosynthetic photon flux density for *L. pumila* was determined. The standard optimal conditions set for measurement of *L. pumila* were 800  $\mu\text{mol}/\text{m}^2/\text{s}$  photosynthetic photon flux density, 400  $\mu\text{mol}/\text{mol}$   $\text{CO}_2$ , 30°C leaf temperature and 60% relative humidity. Photosynthesis measurement was taken at 0800, 1000, 1200, 1400, 1600 and 1800 h, and the daily average photosynthesis was obtained by averaging the six measurements of net photosynthesis taken daily. Photosynthetic light response curves were measured at growth  $\text{CO}_2$  concentrations with an open flow infrared gas analyzer attached with a red LED light source (LI-6400, Li-Cor, Inc., Lincoln, NE, USA). Measurements began with saturating light (1,000  $\mu\text{mol}/\text{m}^2/\text{s}$ ), followed by nine incremental light reductions (900, 800, 700, 600, 500, 400, 300, 200, 100  $\mu\text{mol}/\text{m}^2/\text{s}$ ) until the final irradiance was 10  $\mu\text{mol}/\text{m}^2/\text{s}$ . Assimilation was recorded at each light level following a 15-min acclimation period. Use of decreasing light rather than increasing light reduces the equilibrium time required for stomatal opening and photosynthetic induction (Ibrahim et al. 2010; Kubiske and Pregitzer 1996). Photosynthetic light response curves were individually analyzed to determine the light-saturated net photosynthetic rate ( $A_{\text{max}}$ ), the light compensation point and light saturation point (Ibrahim et al. 2010; Givnish 1988).

Measurements of chlorophyll fluorescence were taken from fully expanded second leaf from the apex. Leaves were darkened for 20 min by attaching light-exclusion clips to the central region of the leaf surface. Chlorophyll fluorescence was measured using a portable chlorophyll fluorescence meter (Handy PEA, Hansatech Instruments Ltd, Kings, Lynn, UK). Measurements were recorded for 5 s (Philip et al. 2006). The fluorescence responses were induced by emitting diodes. Measurement of  $F_o$  (initial fluorescence),  $F_M$  (maximum fluorescence) and  $F_V$  (variable fluorescence) were obtained from this procedure.  $F_V$  is derived as the differences between  $F_M$  and  $F_o$ . The mean value of three representative plants was used to represent each sub-plot.

The microclimate characteristics outside and inside the glasshouse, particularly  $\text{CO}_2$  concentration, photosynthetic photon flux density and temperature, were observed to be largely different (Fig. 1). The  $\text{CO}_2$  concentration in the

glasshouse was found to be higher than that outside at 0800 h (450  $\mu\text{mol}/\text{mol}$ ), but showed a big reduction than that outside at 1000 h (410  $\mu\text{mol}/\text{mol}$ ) and then started to increase again at 1800 h. Conversely,  $\text{CO}_2$  concentration outside was stable throughout the day and ranged from 419 to 425  $\mu\text{mol}/\text{mol}$  (Fig. 1a). Photosynthesis photon flux density (PPFD) peaked at 1400 h in both the open and glasshouse conditions where PPFD for the former condition registered 50% higher PPFD than the glasshouse (1,215 vs. 606  $\mu\text{mol}/\text{m}^2/\text{s}$ ; Fig. 1b). Similarly, the PPFD patterns follow the same trend as the diurnal pattern of temperature. The highest temperature recorded under both growing conditions peaked at 1400 h with open field and



**Fig. 1** Changes with time of the day in  $\text{CO}_2$  concentration (a), photosynthetic photon flux density (b), and temperature (c) of *Labisia Pumila* plants grown in the open field (open diamond with line) and greenhouse (closed square with line) growing conditions,  $n = 27$

greenhouse conditions recording temperatures of 37 and 33°C, respectively (Fig. 1c).

## Results

### Diurnal net photosynthesis

The different growing conditions had significant effects on the photosynthetic patterns of *L. pumila*. In greenhouse, the highest net photosynthesis (*A*) peak was achieved between 1200 and 1400 h (Fig. 2). For plant grown under open

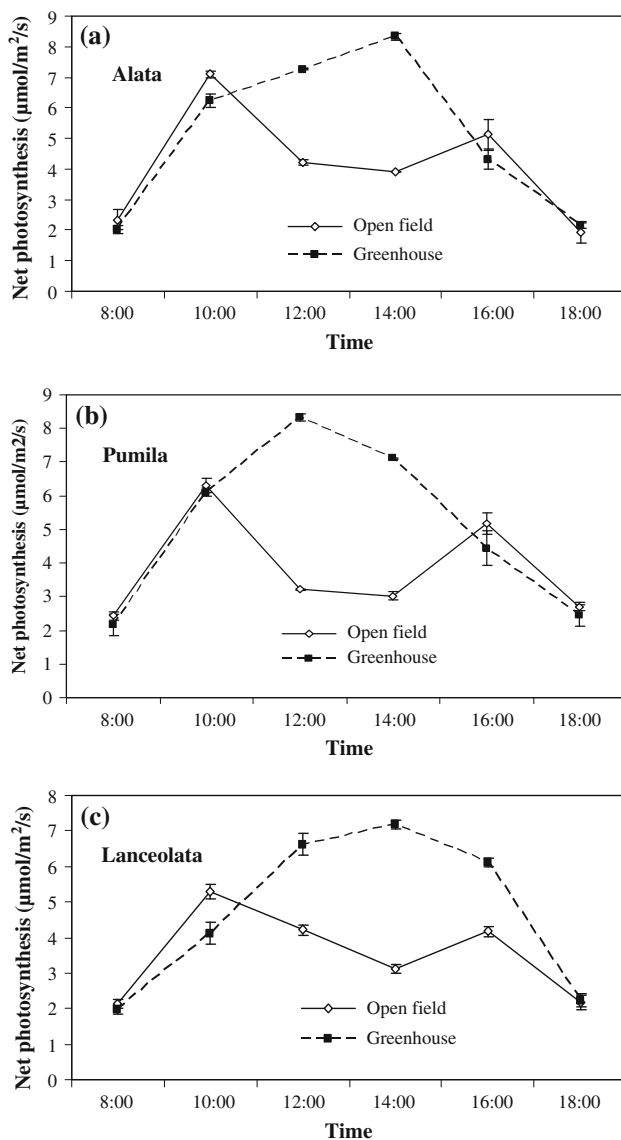
field, there were double peaks observed at 1000 and 1600 h in all the three varieties. Daily average *A* of open field plants were 4.10, 3.81, 3.51  $\mu\text{mol}/\text{m}^2/\text{s}$  and 5.05, 5.02, 4.71  $\mu\text{mol}/\text{m}^2/\text{s}$  in greenhouse for the varieties *Alata*, *Pumila* and *Lanceolata*, respectively. The plants daily *A* was comparatively higher under greenhouse condition compared to the open field by 29.3% (Table 1). Under both growing conditions var *Lanceolata* demonstrated significantly lower daily average as compared to the greenhouse (Fig. 2c), while *A* of var *Alata* recorded higher value (Fig. 2a) than var *Pumila* (Fig. 2b) although they were not significantly different (Table 1). It was also shown that var *Alata* and var *Pumila* acclimatized well under greenhouse condition where the interaction effects between varieties and growing conditions showed that var *Alata* and *Pumila* had the highest daily *A* when planted under greenhouse condition (Table 1).

### Photosynthetic light response curves

Under greenhouse condition, all the three varieties recorded lower light saturation point than those grown in the open field (Table 1). In the latter condition, the light compensation point for var *Lanceolata* was found to be the highest (17.22  $\mu\text{mol}/\text{m}^2/\text{s}$ ), and when treated with the greenhouse conditions the variety exhibited the lowest light light compensation point compared to var *Alata* and var *Pumila* (8.22 vs. 9.12 and 9.72  $\mu\text{mol}/\text{m}^2/\text{s}$ , respectively). It was also observed that the light saturation point was highest in the open area compared to the glasshouse condition where there were no significant differences between all the three varieties. The  $A_{\text{max}}$  measured in the open field condition for all the three varieties displayed lower values than those from the greenhouse with var *Lanceolata* exhibiting the lowest  $A_{\text{max}}$  (6.01  $\mu\text{mol}/\text{m}^2/\text{s}$ ). However, when exposed to greenhouse condition, var *Alata* registered a substantially ( $p < 0.05$ ) higher  $A_{\text{max}}$  (9.32  $\mu\text{mol}/\text{m}^2/\text{s}$ ) than var *Pumila* (8.37  $\mu\text{mol}/\text{m}^2/\text{s}$ ), followed by var *Lanceolata* (8.22  $\mu\text{mol}/\text{m}^2/\text{s}$ ). Interaction effects between varieties and growing condition showed that  $A_{\text{max}}$  for var *Alata* and var *Pumila* was highest when planted under greenhouse condition (Table 1).

### Chlorophyll fluorescence and chlorophyll content

The diurnal pattern of maximum photochemical efficiencies of PSII ( $F_v/F_m$ ) was influenced by the growing conditions ( $p \leq 0.01$ ; Fig. 3). It was found that  $F_v/F_m$  ratio was higher in the greenhouse compared to the open field condition. For every variety, the  $F_v/F_m$  ratio ranged between 0.821 and 0.847 from morning to evening. For each variety, the  $F_v/F_m$  was almost similar in the morning and evening, and the values were close to 0.85. However,



**Fig. 2** Diurnal variations of net photosynthesis of three varieties of *Labisia pumila* viz var *alata* (a), var *pumila* (b), and var *lanceolata* (c) in the open field (open diamond with line) and greenhouse (closed square with line) growing conditions. Data are mean  $\pm$  S.E.,  $n = 9$

**Table 1** Effects of growth conditions and varieties (open field and greenhouse) on photosynthetic parameters of three (*alata*, *pumila*, *lanceolata*) varieties of *Labisia pumila* Benth

Growth condition	Varieties	Daily average photosynthesis ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	LCP ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	LSP ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	$A_{\text{max}}$ ( $\mu\text{mol}/\text{m}^2/\text{s}$ )
Open field	<i>Alata</i>	$4.10 \pm 0.11\text{a/B}$	$12.12 \pm 0.20\text{b/B}$	$820 \pm 27\text{a/A}$	$7.32 \pm 0.68\text{a/C}$
	<i>Pumila</i>	$3.81 \pm 0.14\text{a/B}$	$13.72 \pm 0.21\text{b/B}$	$778 \pm 21\text{b/B}$	$6.22 \pm 0.12\text{b/C}$
	<i>Lanceolata</i>	$3.51 \pm 0.01\text{b/C}$	$17.22 \pm 0.28\text{a/A}$	$752 \pm 11\text{b/B}$	$6.01 \pm 0.17\text{b/C}$
Greenhouse	<i>Alata</i>	$5.05 \pm 0.29\text{a/A}$	$9.12 \pm 0.16\text{a/C}$	$652 \pm 6\text{b/C}$	$9.32 \pm 0.17\text{a/A}$
	<i>Pumila</i>	$5.02 \pm 0.42\text{a/A}$	$9.72 \pm 0.21\text{a/C}$	$700 \pm 12\text{a/C}$	$8.37 \pm 0.52\text{a/A}$
	<i>Lanceolata</i>	$4.71 \pm 0.21\text{b/B}$	$8.22 \pm 0.22\text{b/C}$	$603 \pm 5\text{b/C}$	$8.02 \pm 0.57\text{b/B}$

The values are mean  $\pm$  SEM ( $n = 9$ )

Different letters in each column express significantly different results between growth condition treatments within the same varieties (a and b) or between the varieties within the same treatments (A, B, C) ( $p \leq 0.05$ )

LCP light compensation point, LSP light saturation point,  $A_{\text{max}}$  maximum net photosynthetic rate

the lowest  $F_v/F_m$  ratio was observed around 1400 h under open field condition (0.626, 0.726, 0.526) for var. *Alata*, *Pumila* and *Lanceolata*, respectively. However, under greenhouse at 1400 h, the  $F_v/F_m$  ratio inside the greenhouse changed a little, had frail point and varied between 0.826 and 0.836. No statistical significance was observed in total chlorophyll content under different growth conditions, although chl *b* was higher from plants growing under greenhouse than open field that significantly showed lower chl *a*/chl *b* ratio in greenhouse than in open field (Table 2).

## Discussions

The photosynthetic response of leaves to increased photon flux density (PPFD) depends on an intricate interaction between acclimation of photosynthetic machinery, photoinhibitory damage and repair (Adir et al. 2003; Percy and Sims 1994). The existence of the dual peaks of diurnal *A* curves exhibited by *L. pumila* plants exposed to open field condition might have resulted from photoinhibition under high PPFD condition between 800 and 1200  $\mu\text{mol}/\text{m}^2/\text{s}$  (Matos et al. 1998). The photosynthetic response of mature leaves to increased PPFD depends on the complex interactions between photoinhibitory repair, damage, acclimation and photoprotection of the photosynthetic machinery (Su and Liu 2005). It was also found that a decline in *A* for all the three varieties in open field condition occurred after 1000 h. The decrease might be due to an increase in PPFD that simultaneously escalated temperature outside the greenhouse (Fig. 1; Jaafar 2006). From the daily average of *A* (Table 1), each variety demonstrated similar acclimation trait in the open field as well as in the greenhouse. Var *Alata* and *Pumila* exhibited better adaptation in both open field and greenhouse growing conditions compared to var *Lanceolata*.

*Labisia pumila* seemed to be a shade adapted plant. In the present study, the  $A_{\text{max}}$  of *L. pumila* increased with simultaneous decrease in the compensation point and light saturation point when plants were exposed to greenhouse growing condition with low PPFD, suggesting that these plants were shade loving species (Lambers et al. 1998). According to Kitao et al. (2000) and Patakas et al. (2003), shade adapted plants have the ability to increase  $A_{\text{max}}$  and other energy dissipating mechanism when grown under low light condition compared to the high light condition. Var *Alata* and var *Pumila* were shown to acclimatize well under greenhouse condition. This is shown in Table 1, where interaction effects between growth conditions and varieties have shown that these varieties have the highest daily *A* and  $A_{\text{max}}$  under greenhouse condition, although there were no significant differences between their light compensation and light saturation point.

Chlorophyll fluorescence is frequently used to determine the state of energy distribution in the thylakoid membrane, photoinhibition and quantum efficiency of PSII (Maxwell and Johnson 2000; Mierowska et al. 2008). Under intense sunlight, photochemical impairment was reflected in either the increase in original fluorescence ( $F_o$ ), or in the decrease in maximum fluorescence ( $F_m$ ), or in the ratio of variable,  $F_v$  (equals to  $F_m - F_o$ ), to maximal fluorescence ( $F_v/F_m$ ) (Thomas and Turner 2001). In the current study, the  $F_v/F_m$  ratio decreased as the PPFD increased under both growing conditions, with decrease in the open field being more acute (Fig. 3). The result signified that photoinhibition had occurred under high light state between 1200 and 1400 h reaching the peak at the latter hour regardless of plants' growing conditions. In the open field, photoinhibition was related to the double peaked curves of diurnal *A* during daylight as a result of high PPFD (1200  $\mu\text{mol}/\text{m}^2/\text{s}$ ). Subsequently, rapid recoveries of  $F_v/F_m$  were recorded from all the three varieties exposed to open field condition, which confirmed the presence of photo-defensive

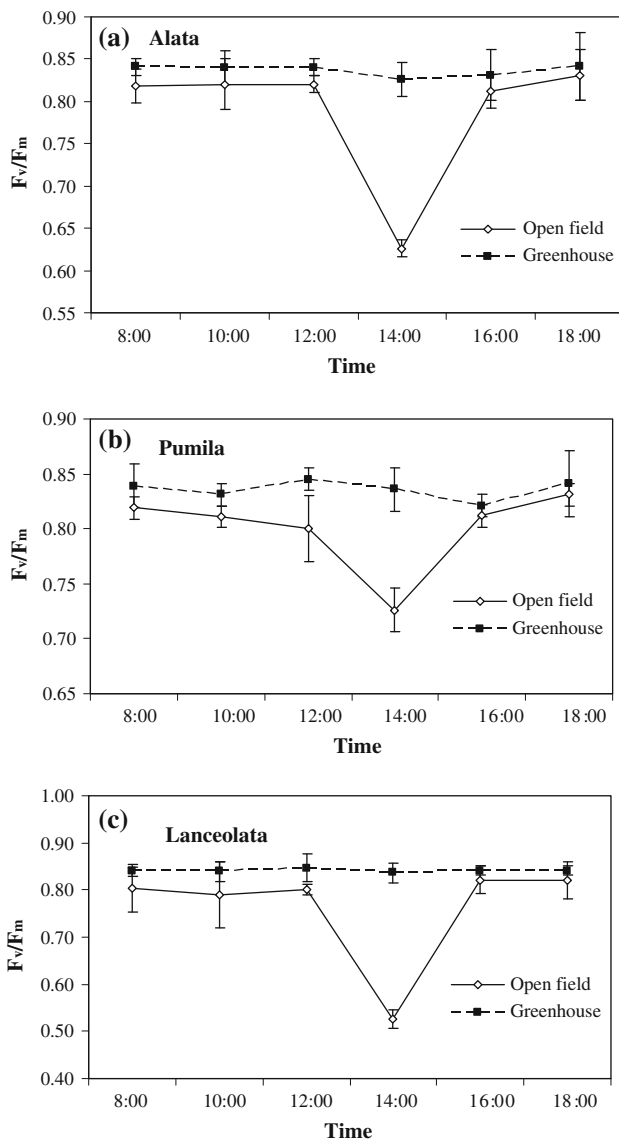


**Table 2** Chlorophyll content of *Labisia pumila* Benth. grown in open field or greenhouse conditions

Growth condition	Chlorophyll content (mg/g fresh weight)			Chl <i>a</i> /chl <i>b</i>
	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i> + <i>b</i>	
Open field	2.67 ± 0.01a	3.77 ± 0.02b	6.70 ± 0.08a	0.77 ± 0.05a
Greenhouse	2.77 ± 0.06a	4.91 ± 0.01a	7.23 ± 0.02a	0.55 ± 0.06b

The means are mean ± SEM ( $n = 18$ )

Different alphabets following the values within columns show significant differences at  $p \leq 0.05$  between growth condition treatments, whereas similar alphabets show non-significant differences between the treatments



**Fig. 3** Diurnal variations of maximal photosystem II efficiency ( $F_v/F_m$ ) of three varieties of *Labisia pumila* viz var *alata* (a), var *pumila* (b), and var *lanceolata* (c) in the open field (open diamond with line) and greenhouse (closed square with line) growing conditions. Data are mean ± S.E.,  $n = 9$

mechanism that might aid photosynthesis recuperation when favorable conditions are restored (Maxwell and Johnson 2000; Hernandez et al. 2004).

In the greenhouse condition, chl *b* was observed to be higher than chl *a*. Plants that have high chl *b* than *a* are generally able to accommodate to the low light situation (Lei et al. 1996). In this study, reduction in the ratio of chl *a*:*b* in the leaves of greenhouse plants with significant increase in chl *b* signified possible changes in the establishment of both light harvesting and electron transport components (Schiefthaler et al. 1997). The lowering of chl *b* could be an indication of chl destruction by excess irradiance under the open field condition (Jason et al. 2004). The result suggested that the high chl *a* to *b* ratio without significant changes in total chl content could be considered as a protecting mechanism due to the consequence of exposing plants to open field condition (Wang et al. 2007). It may be hypothesized that total chl content was high enough to enable the occurrence of high non-photochemical quenching (NPQ) of plants in open field and greenhouse conditions, and this could have facilitated efficient photo fortification of the photosynthetic apparatus against excess irradiation as established in the present study by the photosynthetic recovery observed after the 1400 h (Fig. 2; Thomas and Turner 2001; Patricia et al. 2001).

The current study had indicated that out of the three varieties investigated, var *Alata* and var *Pumila* displayed the highest ability to acclimatize to the greenhouse condition compared to var *Lanceolata*. Hence, they could be the best choice of selection for commercial planting under greenhouses. The approach of acclimation normally involves altered antenna size of photosystem II (Bailey et al. 2001), adjustment in reaction center stoichiometry (Walters and Horton 1994) and distorted levels of Rubisco (Seemann et al. 1987). Such acclimation is readily monitored through the consequent changes in the ratio of chl *a* to chl *b*, and in the light-saturated rate of photosynthesis ( $A_{max}$ ) that had also been established in the present experiment.

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