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海拔对高山栎光合气体交换和叶性状的影响*

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摘要: 理解影响植物分布的式样及过程是生态学研究的中心内容之一, 但对许多物种而言, 限制其分布的原因还不清楚。为了认识高山栎分布与生理生态特性的关系, 我们在不同海拔的 4 个观测点研究了帽斗栎的光合气体交换、叶氮含量、叶绿素含量和比叶重。由于高的水气压亏缺和气温, 帽斗栎的光合作用和蒸腾作用在午间表现出明显的降低现象。帽斗栎的饱和光合速率、水分利用效率、最大羧化速率、最大电子传递速率和氮利用效率在海拔中部比低海拔或高海拔处的为高。不同海拔的叶氮含量在 5 月份有差异, 8 月份则没有明显不同。叶片厚度随海拔增加, 但叶绿素含量及光合最适温度随海拔升高而降低。帽斗栎光合作用的海拔变化与叶片的生化效率和氮含量有关, 而与比叶重无关。研究结果说明, 温度的海拔变化对高山栎的光合作用和叶性状有明显影响, 最适宜帽斗栎光合碳获取及生长的海拔范围是 3 180~3 610 m。

关键词: 帽斗栎; 光合作用; 叶绿素荧光; 叶性状; 高山生境; 生态适应
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Effects of Altitude on Photosynthetic Gas Exchange and the Associated Leaf Trait in an Alpine Oak, *Quercus guyavifolia* (Fagaceae)

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Abstract Understanding the pattern and process governing the distribution is a central goal of ecology, yet for many species the causes of distribution limit are unknown. To understand the relationship between altitudinal distribution of alpine oak and ecophysiological trait, leaf nitrogen content, chlorophyll content, leaf mass per unit area and photosynthetic gas exchange of *Quercus guyavifolia* were investigated at four sites along an altitudinal gradient from 2 650 to 3 920 m in the Hengduan Mountains. *Q. guyavifolia* showed a significant midday depression in photosynthesis and transpiration at all sites due to high vapour pressure deficit and temperature. Both in May and August, this species had higher light-saturated photosynthesis, water use efficiency, maximum RuBP rate of carboxylation, light saturated rate of electron transport and photosynthetic nitrogen use efficiency at the middle altitude than at the lowest or highest location. Leaf nitrogen content was different in May among altitudes, but remained relatively constant in August. Leaf thickness increased with altitude while chlorophyll content and photosynthetic optimum temperature decreased. The altitudinal trend in photosynthesis of *Q. guyavifolia* could be linked to leaf biochemical efficiency and nitrogen content, but not leaf mass per unit area. The variation in temperature along the altitudinal gradient imposed a con-

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straint on photosynthesis and leaf trait. The altitudinal range from 3 180 m to 3 610 m would be optimal for the photosynthetic carbon gain and growth of *Q. guyavifolia*.

Key words *Quercus guyavifolia*; Photosynthesis; Chlorophyll fluorescence; Leaf traits; Alpine environment; Ecological adaptation

Introduction

Every species is limited both geographically and ecologically to a range of available habitats. Understanding the pattern and process governing the distribution is a central goal of ecology, yet for many species the causes of distribution limit are unknown (Angert, 2006). This question is particularly important today because of the potential sensitivity of distribution boundary of tree to climate change (Brodersen *et al*, 2006; Lenoir *et al.*, 2008). Species′ altitudinal range limit may, in part, be due to metabolic limitation on growth that ultimately decreases survival and limits reproduction (Angert, 2006), because altitudinal change in environments has an important effect on plant physiology and morphology (Hovenden and Brodribb, 2000). Previous studies showed that leaf thickness, leaf nitrogen content and photosynthetic capacities of alpine plants are higher than those of lowland plants (Hultine and Marshall, 1999; Cordell *et al*, 1999; Qi *et al.*, 2007). However, the contradictory data provided by several authors (Rada *et al.*, 1998; Cabrera *et al.*, 1998; Bowman *et al.*, 1999) showed that there is no general trend in photosynthesis across altitudes, as the altitudinal variation in photosynthesis can be caused by multiple factors, such as environmental condition, genetic trait of plant, leaf anatomy and physiology. It is believed that alpine environments are very sensitive to global changes, but it is unclear whether alpine plants are sensitive to global change. Consequently, the physiological ecology, and particularly the leaf gas exchange of plant at high altitude, has attracted increasing attention (Pelfini *et al.*, 2006). However, data on the physiological ecology of plants over altitude of 3 000 m is limited (Li *et al.*, 2006; Zhang *et al.*, 2007), especially for the alpine plants in the Hengduan Mountains, located at the eastern end of the Himalayan range (Li *et al.*, 2006). Consequently, little is known about the altitudinal patterns in ecophysiological traits of alpine plants at high elevations, and how alpine plants adapt to their environments in the Hengduan Mountains. This information is essential for understanding the rich diversity of species in the Hengduan Mountains and predicting the response to alpine plants to climate change.

Quercus sect. *Heterobalanus*, distributed from alt. 1 700–4 800 m, is the dominant component of evergreen sclerophyllous oak forests in the Hengduan Mountains, and plays an important role in preventing soil erosion and water loss, as well as in maintaining ecological stability (Zhou *et al.*, 2003). Their large ranges of habitats across different elevations imply a strong adaptation to different environments, and would be beneficial for understanding the relationship between altitude and ecophysiological trait of plant.

Usually, evergreen sclerophyllous oaks occur in the xerothermic zone of the world, but the oaks of *Quercus* Sect. *Heterobalanus* are distributed in the cold and moist habitats of the Hengduan Mountains, and there still remain obvious xerophytic characters, such as dense hairs and low stomatal density (Zhou *et al.*, 2003). Temperature is thought to be one of the primary determinants of species distribution and growth along altitudinal gradients (Cabrera *et al.*, 1998; Körner, 1998). Previous studies suggested that *Quercus* Sect. *Heterobalanus* can adapt to alpine environments due to their xerothermic characters and unique genetic structure, and the altitudinal ranges from 2 400 m to 3 600 m are their optimum distribution zone (Zhou *et al*, 2003). Zhang *et al.* (2005) showed that photosynthetic capacity of *Q. pannosa* decreases from 3 240 m to 4 170 m in the Hengduan Mountains, while Li *et al.* (2006) suggested that near 2 800 m altitude is the optimum zone for growth of *Q. aquifolioides*. Obviously, the physiological mechanisms concerning the altitudinal distribution of alpine evergreen sclerophyllous oak in the Hengduan Mountains have not been studied sufficiently, and relevant data are fragmentary.

The photosynthesis and leaf traits of *Q. guyavifolia* were studied at four sites of different altitudes in the Hengduan Mountains. The aims were (i) to characterize the photosynthetic adaptation of alpine evergreen sclerophyllous oak to environment; (ii) to investigate the differences in chlorophyll content, leaf N content and leaf mass per unit area affecting photosynthetic performance along the altitudinal gradient; (iii) to understand the relationship between altitudinal distribution of *Q. guyavifolia* and photosynthetic gas exchange and leaf trait.

Materials and methods

Material and study sites

The research was conducted at four sites along an altitudinal gradient in the Hengduan Mountains of southwestern China: site A (99°26.69′E, 28°07.57′N), site B (99°34.90′E, 27°57.99′N), site C (99°36.81′E, 27°56.03′N) and site D (99°39.77′E, 27°53.01′N) at altitudes of 2 650, 3 180, 3 610 and 3 920 m respectively. As the atmospheric pressure deceases with increasing elevation, the partial pressure of CO_2 at site A, site B, site C and site D are 25.8, 24.2, 22.9 and 21.9 Pa, respectively. The long-term climatic data of study sites are unavailable, but the climatic data in Zhongdian weather station nearby site B is available. The air temperatures at study sites were calculated from the altitudinal lapse rate of 7.1℃/1 000 m in this region (Zhang 1998). The annual pattern of temperature was given in Fig. 1. May to October is the rainy season with 87% of annual rainfall, while the dry season occurs from November to April. The soil at all four sites are brown soils with pH values of 6.2–6.9.

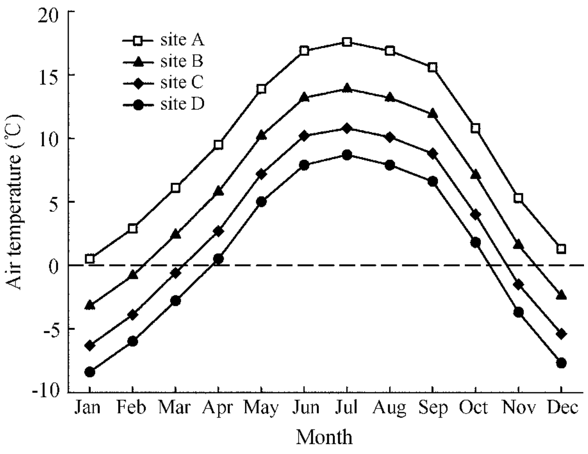

Fig. 1 Seasonal variations of air temperature at four study sites. Based the climatic data from Zhongdian weather station nearby site B, the air temperatures at study sites were calculated from the altitudinal lapse rate of 7.1℃/1 000 m in the Hengduan Mountains

Quercus guyavifolia L vl. is an evergreen broadleaf tree that occurs in the mountain oak forests or pine-oak mixed forests at altitudes between 2 500 m and 4 000 m in southwestern China. The new leaves emerge from April to May, and are retained for 2–3 years. The trees blossom from April to May and their fruits ripen between October and November. Observations at four open sites were conducted in May and August 2003. Five trees of 4–5 m height of *Q. guyavifolia* were selected at each site for measurements.

Measurement of photosynthesis and chlorophyll fluorescence

Diurnal gas exchange variations were measured in May 2003. Five fully expanded leaves from the upper position facing east of five sampling trees per site were selected from sampling trees for hourly measurements from 08:00 h to 19:00 h on the clear days in May 17–20, 2003. Photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 partial pressure (C_i) were recorded using a portable infrared gas analyser with a leaf chamber type PLC-B (CIRAS-1, PP Systems, UK) under ambient conditions. Meanwhile, a Li-1400 data logger (Li-Cor, NE, USA) was used to record irradiance and air temperature at 1-hour interval. Leaf and air temperature and relative humidity were used to calculate leaf-to-air vapour pressure deficit (VPD). Water use efficiency (WUE) was calculated as the ratio between A and E.

The photosynthetic responses to CO_2, light and temperature were measured both in May and August 2003. Photosynthetic responses to photosynthetic photon flux density (PPFD) were measured by using a CIRAS-1 infrared gas analyser at ambient CO_2 partial pressure and 20℃ leaf temperature. After the initial measurement at 2 000 $\mu mol\ m^{-2}\ s^{-1}$, PPFD was decreased to produce 13 subsequent levels at which photosynthetic rates were recorded. Data were fit by a non-rectangular hyperbola (Prioul and Chartier, 1977). Using this function, apparent quantum efficiency (AQE) and light-saturated photosynthetic rate (A_{max}) were estimated by Photosyn Assistant software (v1. 1, Dundee Scientific, UK).

Following A–PPFD curves, the CO_2 responses of photosynthesis were determined with a range of CO_2 partial pressure at PPFD of 1 200 $\mu mol\ m^{-2}\ s^{-1}$ and 20℃. CO_2 was injected into the circuit using the built-in injection system of the gas analyser. After the initial measurements at ambient partial pressure, CO_2 partial pressure was reduced to 0 Pa and then increased in steps to produce CO_2 response curves. Using A–C_i curves, the maximum carboxylation rate by Rubisco (V_{cmax}) and light-saturated electron transport (J_{max}) were calculated by Photosyn Assistant software that applied the biochemical model of von Caemmerer and Farquhar (1981). The intercellular CO_2 partial pressures at different altitudes were calibrated according to the differences in air pressure. Relative stomatal limitation (L_s) of photosynthesis, an estimate of proportion of the reduction in photosynthesis attributable to CO_2 diffusion from atmosphere to intercellular space, was calculated from A–C_i curves using the method of Farquhar and Sharkey (1982).

The dependence of photosynthesis on temperature was examined with five fully expanded leaves from five sampling trees using an infrared gas analyser between 08: 00 h and 11: 00 h in the morning. Leaf temperature was adjusted using the internal heating/cooling system. Measurements were made between 10℃ and 35℃ at ambient CO_2 partial pressure and PPFD 1 200 $\mu mol\ m^{-2}\ s^{-1}$. After the initial measurements at 20℃, leaf temperature was reduced to 10℃ and then increased to 35℃. Each temperature maintained 5 min. A second-order polynomial equation was used to fit the photosynthetic optimum temperature (T_{opt}).

Five expanded leaves per site were selected from sampling trees for hourly measurements from 08: 00 h to 19: 00 h. Chlorophyll fluorescence was measured on dark-acclimated leaf (30 min) with a FMS-2 pulse modulated fluorometer (Hanstech, Norfolk, UK). After the minimal fluorescence (F_o) was determined by a weak modulated light, A 0.8 s saturating light of 8 000 $\mu mol\ m^{-2}\ s^{-1}$ was used to determine the maximal fluorescence (F_m). Then the leaf was illuminated by an actinic light of 1 200 $\mu mol\ m^{-2}\ s^{-1}$. After 5 min, the steady-state fluorescence (F_s) was recorded and a second 0.8 s saturating light of 8 000 $\mu mol\ m^{-2}\ s^{-1}$ was given to determine the maximal fluorescence (F_m') on the light-acclimated leaf. The fluorescence parameters were calculated as $F_v/F_m = (F_m - F_o)/F_m$ and $\Phi_{PSII} = 1 - F_s/F_m'$. The second-degree polynomial equation was used to assess the relationship between temperature and chlorophyll fluorescence.

Leaf traits

Twenty leaves nearby the leaves used in photosynthetic measurements were harvested from the upper part of sampling trees. In the laboratory, leaf areas were measured using a Li-3000A leaf area meter (Li-Cor, NE, USA), and then the leaves were dried to a constant mass at 70℃ for 48 h to measure the dry mass and calculate leaf mass per unit area (LMA). The nitrogen concentration of these leaves were analysed using a Leco FP-428 CHN analyser (Leco Corporation, MI, USA). Leaf nitrogen content per unit area (N_a) was calculated by leaf N concentration per unit mass multiplying LMA. Photosynthetic nitrogen use efficiency (PNUE) was calculated as the ratio between A_{max} and N_a. Chlorophyll content was extracted from 20 leaf disk (0.38 cm^2 per disk) on the leaves nearby the the leaves used in photosynthetic measurements in the sampling trees with N, N-Dimethylformamide, and stored in the dark at 4℃ for 5– 7 days. Chlorophyll content was analyzed with a UV-2550 spectrophotometer (Shimadzu, Japan) and calculated using the method of Inskeep and Bloom (1985).

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, USA). Differences in leaf morphological and physiological variables among altitudes were determined using one-way analysis of variance (ANOVA) and LSD test for multiple comparisons, and between May and August by independent samples t-test.

Results

Diurnal of environmental factors and gas exchange

At all sites, the maximum PPFD, temperature and VPD were observed around 14:00 h (Fig. 2). There was no statistical difference in average daily PPFD among altitudes ($F = 0.503$, $P > 0.05$). However, both air temperature ($F = 11.868$, $P < 0.001$) and VPD ($F = 4.894$, $P < 0.01$) decreased as altitude increased.

The maximum g_s occurred in the early morning, decreased towards midday and increased in the afternoon (Fig. 3). The diurnal variation of g_s was similar to that of relative humidity (data not presented), an opposite trend to VPD. Although the g_s values of plants at the altitudes of 3 180 m and 3 610 m were higher than at the altitudes of 2 650 m and 3 920 m, there was no significant difference ($F = 1.482$, $P > 0.05$).

Diurnal variations of E and A of *Q. guyavifolia* showed a significant midday depression. The minimum value of E was observed around noon, and E decreased with increasing altitude. The average A of 5 leaves at all sites peaked rapidly after dawn, before subsiding in the middle of the day, and reached maximum values at about 10:00 h, then reached a second-peak in the late afternoon (Fig. 3). The plants had higher daily mean photosynthetic rate at the altitudes of 3 180 m and 3 610 m than at the altitudes of 2 650 m and 3 920 m ($F = 3.173$, $P < 0.05$). The diurnal variation of WUE was similar to A, and the plants at altitude of 3 610 m had the highest WUE among altitudes ($F = 8.015$, $P < 0.001$).

Effects of light and temperature on photosynthesis

The photosynthesis of *Q. guyavifolia* was saturated around the light intensity of 1 000 μmol m^{-2} s^{-1} (Fig. 4). There was no significant difference in light compensation point among sites in August ($F = 0.574$, $P > 0.05$), but the plants at altitude of 3 610 m in May had lower light saturation points than at other three sites ($F = 9.934$, $P < 0.01$). The photosynthetic

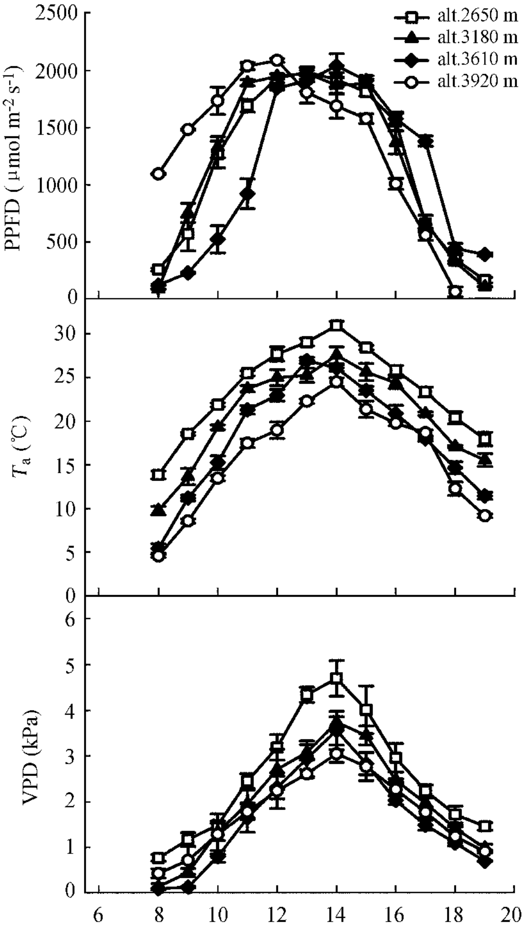

Fig. 2 Diurnal variations of photosynthetic photon flux density (PPFD), air temperature (T_a) and leaf-to-air vapor pressure deficit (VPD) on the clear day in May 2003. Data are means ± 1SE ($n = 5$)

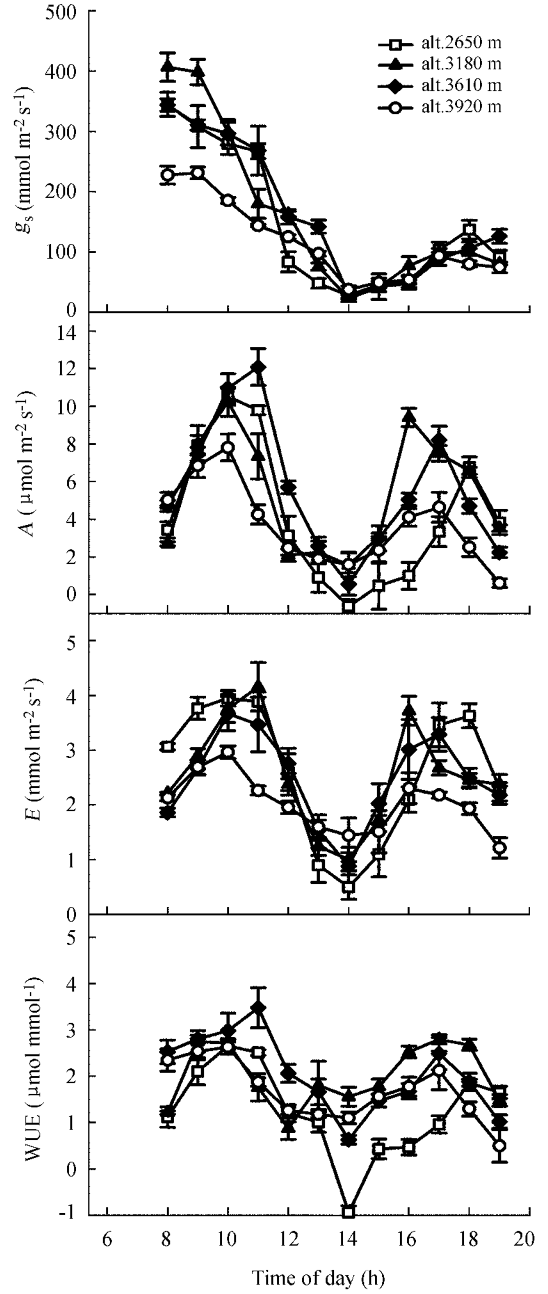

Fig 3 Diurnal patterns of stomatal conductance (g_s), transpiration rate (E), net photosynthesis (A) and water use efficiency (WUE) of *Q. guyavifolia* at ambient temperature, light intensity and CO_2 partial pressure in May 2003. Each point is a mean of 5 measurements Bars represent ±1 SE

light saturation point at altitude of 3 920 m was higher than at other three sites in May ($F = 19.954$, $P < 0.001$) or August ($F = 6.489$, $P < 0.05$).

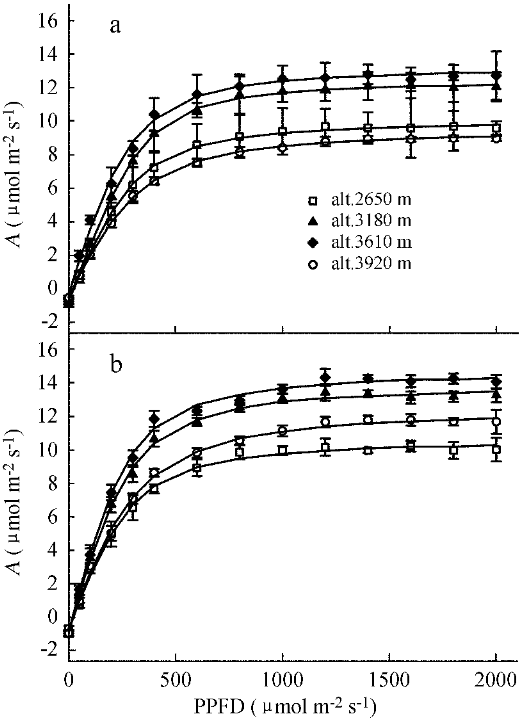

Fig 4 Photosynthetic responses of *Q. guyavifolia* to photosynthetic photon flux density (PPFD) at ambient CO_2 partial pressure and leaf temperature of 20℃ in May (a) and August (b). Vertical bars indicate standard errors of means for five measurements

The optimum temperatures for photosynthesis (T_{opt}) of *Q. guyavifolia* were between 18℃ and 22℃ at all sites in May, and between 20℃ and 23℃ in August T_{opt} decreased with the increasing altitude both in May and August (Fig 5). The T_{opt} of *Q. guyavifolia* in August were higher than in May at all sites The temperature range attaining above 90% A_{max} was reduced with increasing altitude There was a drastic decrease in photosynthesis when leaf temperature was greater than 25℃. The data of chlorophyll fluorescence of *Q. guyavifolia* at all sites was analysed together using a second-degree polynomial equation to address the relationship between chlorophyll fluorescence and temperature (Fig 6). Between 18℃ and 22℃, *Q. guyavifolia* had highest F_v/F_m and Φ_{PSII} values

Altitudinal changes in photosynthetic capacity

The light-saturated photosynthesis (A_{max}) of *Q. guyavifolia* at the middle attitudes (alt 3 180m and alt 3 610m) was higher at the upper altitude (alt 3 920 m) and lower altitude (alt 2 650 m) in both May and August (Table 1). From May to August,

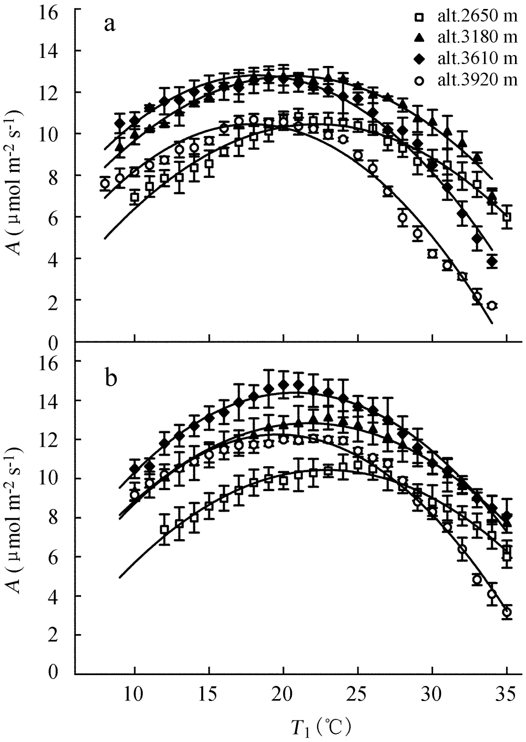

Fig. 5 Effects of leaf temperature on photosynthesis of *Q. guyavifolia* at ambient CO_2 partial pressure and photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1} in May (a) and August (b). Vertical bars indicate standard errors of means for five measurements

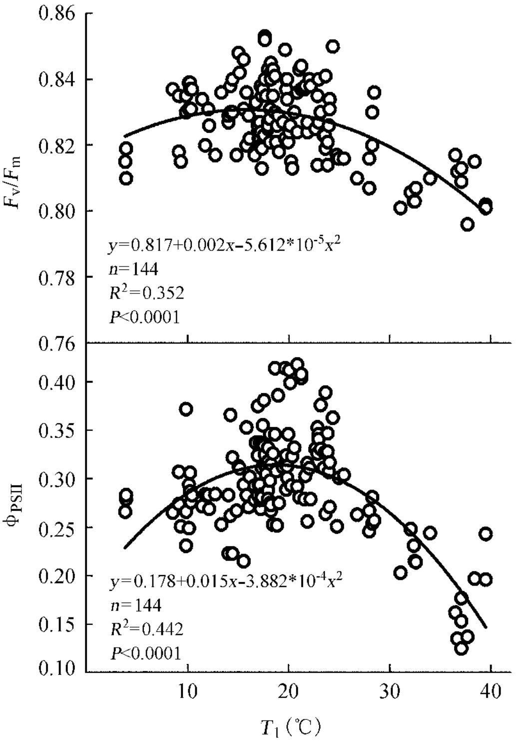

Fig. 6 Effects of temperature on maximum photochemical efficiency (F_v/F_m) and quantum yield of PSII (Φ_{PSII}) of *Q. guyavifolia* across all the trees and all the sites

Table 1 Comparison of photosynthetic parameters of *Quercus guyavifolia* at different altitudes and seasons

		Altitude (m)				
		2650 m (site A)	3180 m (site B)	3610 m (site C)	3920 m (site D)	*p*
A_{max}	May	10.93±0.43^a	13.27±0.55^b	13.77±0.58^b	10.13±0.43^a	**
	August	11.70±0.45^a	14.77±0.55^{bc}	15.63±0.75^b	13.47±0.66^{ac}	**
	p	ns	ns	ns	*	
AQE	May	0.031±0.003^a	0.035±0.001^{ab}	0.041±0.002^b	0.027±0.002^a	**
	August	0.033±0.001^a	0.045±0.002^b	0.051±0.003^b	0.038±0.002^a	***
	p	ns	*	ns	**	
V_{cmax}	May	34.23±1.53^a	39.03±2.40^{ab}	44.57±1.30^b	34.73±1.36^a	**
	August	36.73±1.30^a	41.77±1.87a	51.73±2.87^b	36.37±2.12^a	**
	Sig.	ns	ns	ns	ns	
J_{max}	May	103.07±4.33^a	111.07±4.27^{ab}	121.07±4.22^b	89.33±5.60^a	**
	August	119.33±5.18^a	130.67±3.71^a	150.67±7.97^b	120.33±5.55^a	*
	p	ns	*	*	*	
J_{max}/V_{cmax}	May	3.01±0.01^a	2.85±0.07^b	2.71±0.02^b	2.56±0.06^g	***
	August	3.25±0.07^a	3.14±0.11^a	2.91±0.02^a	3.32±0.10^a	ns
	p	ns	ns	**	**	
L_s	May	30.51±1.62^a	19.55±0.70	23.88±1.33^c	24.29±0.24^c	***
	August	18.43±0.24^{ab}	21.07±0.93^{ab}	14.69±3.18^b	22.51±2.19^a	*
	p	**	ns	ns	ns	

Data are means ± 1SE. Significance: ns, no significant difference; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The same letters in a row indicate no significant difference. A_{max}, light-saturated photosynthetic rate (μmol m^{-2} s^{-1}); AQE, apparent quantum efficiency (mol CO_2 mol^{-1} photon); V_{cmax}, maximum RuBP saturated rate of carboxylation (μmol m^{-2} s^{-1}); J_{max}, light saturated rate of electron transport (μmol m^{-2} s^{-1}); L_s, relative stomatal limitation (%)

A_{max} increased at all sites, but the increment at the upper altitude was the highest among four sites. The altitudinal trend in AQE of *Q. guyavifolia* was similar to that of A_{max}.

Photosynthetic parameters from the A–C_i curves also suggested that the photosynthetic capacity of *Q. guyavifolia* at the middle altitudes were higher than at the upper and lower altitude (Fig. 7 and Table 1). Except for the plants at the lowest altitude (alt. 2 650 m), J_{max} of the plants at other three sites were lower in May than in August, but there were no statistical differences in V_{cmax} between in May and August at all sites. The ratio of J_{max} to V_{cmax} decreased with increasing altitude in May, but they were not significantly different among altitudes in August. The plants in May had lower J_{max}/V_{cmax} ratio than in August.

Altitudinal and seasonal trends in leaf traits

High-altitude *Q. guyavifolia* had lower chlorophyll content than those at lower altitudes (Table 2). The chlorophyll contents of plants in May were lower than those in August. LMA of *Q. guyavifolia* increased with increasing altitude. However, leaf N content per unit area (N_a) at altitude of 3 610 m was higher than at other sites in May, but there was no significant difference among altitudes in August. *Quercus guyavifolia* at lower altitudes had higher PNUE that those at the upper and lower altitude.

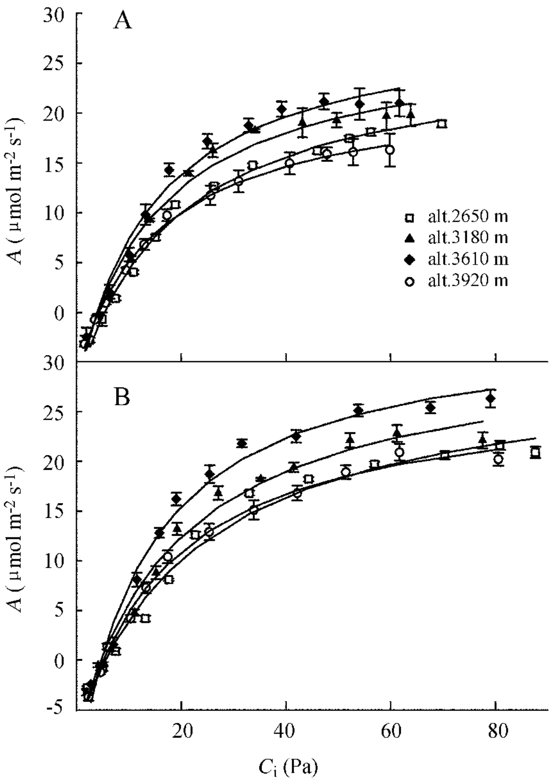

Fig. 7 Photosynthetic responses of *Q. guyavifolia* to intercellular CO_2 partial pressure (C_i) at photosynthetic photon flux density of 1 200 μmol m^{-2} s^{-1} and leaf temperature of 20℃ in May (A) and August (B). Vertical bars indicate standard errors of means for five measurements

Table 2 Comparison of leaf traits of *Quercus guyavifolia* at different altitudes and seasons in the Hengduan Mountains

		Altitude (m)				
		2 650 m (site A)	3 180 m (site B)	3 610 m (site C)	3 920 m (site D)	*p*
Chl	May	40.48±0.83^a	35.45±1.47^b	31.03±1.41^{bc}	27.18±1.02^c	***
	August	44.58±3.96^a	40.96±2.36^a	36.44±1.98^{ab}	33.77±2.54^b	ns
	p	ns	ns	ns	ns	
LMA	May	215.5±6.4^a	218.2±9.9^a	250.8±7.7^b	253.3±12.9^b	*
	August	207.5±4.5^a	202.5±5.2^a	239.6±7.9^b	243.6±11.5^b	*
	p	ns	ns	ns	**	
N_a	May	2.533±0.117^a	2.687±0.167^{ab}	2.893±0.348^b	2.628±0.207^{ab}	*
	August	2.467±0.122^a	2.883±0.136^b	2.997±0.268^b	2.863±0.077^b	ns
	p	ns	ns	ns	ns	
PNUE	May	4.320±0.143^{ab}	4.960±0.168^b	4.803±0.366^b	3.887±0.195^a	*
	August	4.773±0.349^a	5.127±0.052^{ab}	5.260±0.619^b	4.701±0.127^a	*
	p	ns	ns	ns	*	

Data are means±1SE. Significance: ns, no significant difference; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The same letters in a row indicate no significant difference. Chl, chlorophyll content per unit area (μg cm^{-2}); LMA, leaf mass per unit area (g m^{-2}); N_a, leaf N content per unit area (g m^{-2}); PNUE, photosynthetic N use efficiency (μmol CO_2 g^{-1} s^{-1} N)

Discussion

Diurnal variation in photosynthesis

In the present study, *Quercus guyavifolia* experienced a pronounced midday depression in photosynthesis. This was similar to the responses reported for Mediterranean oaks *Q. suber* and *Q. ilex* (Tenhunen *et al.*, 1984). The photosynthetic rate of *Q. guyavifolia* did not substantially decrease under high PPFD conditions, provided temperature was favourable (Fig. 4). When leaf temperature went over 25℃, which typically occurred from 11:00 h to 15:00 h, the photosynthesis of *Q. guyavifolia* decreased dramatically. The inactivation of photosynthesis can be induced by high temperature (Berry and Björkman, 1980). Present study also provided evidence for the important role of high temperature on photosynthetic depression of *Q. guyavifolia* at midday.

Seasonal variation in photosynthesis

In this study, the T_{opt} of *Q. guyavifolia* not only shifted by about 1℃ – 3℃ higher from May to August, but also decreased with increasing altitude irrespective of seasons. The variation in growth temperature can cause a shift in the optimum temperature of photosynthesis (T_{opt}), which allows plants to perform more efficiently at new growth temperatures (Battaglia *et al.*, 1996). This change in optimal temperature would be related to the change in the temperature dependence of Rubisco activity as RuBP carboxylation and RuBP regeneration have different temperature dependence (Ishikawa *et al.*, 2007).

Seasonal changes in environmental factors had a significant effect on photosynthetic capacity. At all sites, the parameters describing photosynthetic capacity in August were higher than those in May (Table 1). Previous study suggested that the change in the ratio of J_{max}/V_{cmax} would be responsible for some parts of seasonal changes in photosynthesis. However, the response of the J_{max}/V_{cmax} ratio to temperature was different among species (Onoda *et al.*, 2005). The J_{max}/V_{cmax} ratio of *Q. guyavifolia* increased with growth temperature which indicated the relative proportion of J_{max} in the photosynthetic proteins decreased under low-temperature conditions. The reduction in J_{max} indicated that the photosynthetic electron transport of *Q. guyavifolia* in May and at the high-altitude sites would be limited by cold stress.

Altitudinal patterns in photosynthesis and leaf traits

This study showed that *Q. guyavifolia* growing at altitudes of 3 180 m and 3 610 m displayed higher photosynthetic capacity (A_{max}) than at altitudes of 3 920 m and 2 650 m. Angert (2006) suggested that two *Mimulus* species attain the greatest biomass, photosynthetic rate and effective quantum yield of PSII when grown under temperature characteristics of the altitudinal range centre. The highest A_{max} of *Pinus sylvestris* is found in the middle parts of the distribution and decreased towards both ends of the transect (Luoma, 1997). Previous studies showed that leaf N content (N_a) in *Metrosideros polymorpha* increased from sea level to treeline (Cordell *et al.*, 1999), but leaf N content per unit area in seven populations of *Frasera speciosa* did not change with altitude along a 1 700 m gradient (Bowman *et al.*, 1999). For *Q. guyavifolia*, the plants had higher N_a at the middle altitude than at the lower and upper altitude in May, but N_a remained relatively constant in August. The photosynthetic N use efficiencies at the middle altitudes were higher than at the upper and lower altitude. Altitudinal variation of N_a appeared to be largely attributable to variation in LMA (Körner and Diemer, 1987), but the LMA of *Q. guyavifolia* was not correlated with N_a and A_{max}. Another reason was that plants with higher LMA limited the supply of CO_2 to chloroplast site because the diffusive path in thicker leaf became longer (Kao and Chang, 2001).

The g_s of *Q. guyavifolia* at altitudes of 3 180 m and 3 650 m were higher than those at altitudes of 2 650 m and 3 920 m. However, Kumar *et al.* (2005) found that the stomatal conductance increases with altitude. According to the data from A–C_i response curves, photosynthetic rate of *Q. guyavifolia* was limited likely by stomatal limitation, since the temperatures at different elevations dramatically affects CO_2 diffusion and the ratio of chloroplast CO_2 partial pressure to ambient CO_2 partial pressure (Shi *et al.*, 2006).

Several authors suggested that temperature is likely to be critical for the limitation on growth, carbon balance, resource usage and reproduction (Körner, 1998; Cavieres *et al.*, 2000). Zhou *et al.* (2003) suggested that sclerophyllous oaks have the highest species richness in the altitudinal range from 2 400 and 3 600 m. *Q. aquifolioides* has higher genetic variability at altitudes of 2 400 – 3 300 m (Li *et al*, 1998). In this study, the plants of *Q. guyavifolia* at the middle altitudes had higher CO_2 assimilation rate than at lower altitude and higher altitude. In the optimum distribution range, alpine oaks have higher resource use efficiency (Zhang *et al.*, 2007). By contrary, the unfavourable environments at the low or high altitudes would limit carbon assimilation, growth and survival of plants (Zu *et al.*, 1998).

Comparison of ecophysiological traits of *Q. guyavifolia* with Mediterranean oaks

Usually sclerophyllous oaks are distributed in the xerothermic regions, but *Q. guyavifolia* occurs in the relatively cold habitats in the Hengduan Mountains. Morphological and genetic evidences suggested that alpine evergreen sclerophyllous oaks in the Hengduan Mountains have closely phylogenetic relationship with Mediterranean oaks (Zhou *et al.*, 2003). Present study showed that the photosynthetic capacity and WUE of *Q. guyavifolia* were similar to those of Mediterranean oaks, but the latter had a lower LMA (Gratani *et al.*, 2000). LMA of *Q. ilex* was higher in the colder sites (Ogaya and Peñuelas, 2007). We speculated that lower temperature in the Hengduan Mountains reduced leaf extension of *Q. guyavifolia* and resulted in thicker leaves.

Compared with the result of Gratani *et al* (2000), the T_{opt} of *Q. guyavifolia* was lower than that of Mediterranean oak (18℃ – 23℃ vs 25℃ – 30℃). *Q. semicarpifolia*, another alpine oak in the Hengduan Mountains, can resist temperature down to − 15℃ (Sakai, 1981). The photosynthetic adaptation of *Q. guyavifolia* to low temperature could be confirmed by fluorescence analysis, as F_v/F_m and Φ_{PSII} can be used as the sensitive indicators of plant photosynthetic performances (Maxwell and Johnson, 2000). Both F_v/F_m and Φ_{PSII} of *Q. guyavifolia* were higher between 18℃ and 22℃, and deceased above 25℃ rapidly. This indicated that *Q. guyavifolia* is well adapted to low temperature rather than high temperature.

In conclusion, altitude had an important effect on leaf morphology and physiology of *Q. guyavifolia*. LMA increased with altitude while chlorophyll content decreased. The highest A_{max} were found at middle altitude. This altitudinal trend in photosynthesis may be linked to biochemical efficiency and N_a. The altitudinal range from 3 180 to 3 610 m would be optimal for the photosynthetic carbon gain and growth of *Q. guyavifolia*. These results would be beneficial for understanding the relationship between altitudinal distribution of alpine oak and ecophysiological traits.

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