Photosynthetic capacity of *Miscanthus* species at high and low elevations of sub-tropical Taiwan through different seasons and the relationships with chlorophyll content and stomatal conductance

Jen-Hsien Weng[1*], Shau-Lian Wong[2], Chung-Wei Chen[3] and Li-Te Feng[4]

[1] China Medical University, Taichung, Taiwan.

[2] Endemic Species Research Institute, Jiji, Nantou, Taiwan.

[3] Yuchi Branch, Tea Research and Extention Station, Yuchi, Nantou, Taiwan.

[4] Department of Life Science, National Chung-Hsing University, Taichung, Taiwan.

* Corresponding author: jhweng@mail.cmu.edu.tw

Received: October 12, 2017; Accepted: June 28, 2018

Abstract

Net photosynthetic rate (P_n) of *Miscanthus* leaves, with different chlorophyll content (*Chl*), was measured at high (natural habitat-grown *M. transmorrisonensis* and *M. sinensis* var. *formosanus* at 3,000 and 2,100 m a.s.l., respectively) and low (pot-grown *M. floridulus* at 70 m a.s.l.) elevations through different seasons. In the winter, even the leaf temperature (T) drops to 6 °C, the P_n of *M. transmorrisonensis* leaves (*Chl* ranged 0.17–0.46 g m^{-2}) that were measured under the photosynthetic photon flux density (PPFD) of 1,200 μmol m^{-2} s^{-1} (denoted as P_{1200}) could retain 0.4–3.2 μmol CO_2 m^{-2} s^{-1}. While in the summer, the P_{1200} measured at 19 °C could raise to 3.9–19.9 μmol CO_2 m^{-2} s^{-1}. At low elevation, *M. floridulus* could raise its P_{1200} and P_{2000} to the values about 30 and 40 μmol CO_2 m^{-2} s^{-1}, respectively, at both 25 and 30 °C. At the same T and PPFD, leaves with higher *Chl* always had higher P_n and stomatal conductance (g_s). Yet, the determination coefficient of P_n–g_s relationship always higher than that of P_n–*Chl*, and the slopes of P_n–g_s relation were closely related to T. Thus, a significant positive linear regression ($P_n = 1.147 + 4.222 \bullet g_s \bullet T$) could be fitted by combining the data measured at different elevation, season and PPFD. The P_n estimated from this equation was closely related to the measured P_n (regression line approximate to 1:1 line, r^2=0.937, P<0.001). For verifying this equation furthermore, the g_s data of the pot-grown *M. floridulus* at 250 m a.s.l. (from Wong et al., 2014) were conducted to compute the estimated P_n and the results still showed that the estimated value could still correlate to the measured strongly (r^2=0.856, P<0.001). Due to g_s and T could be determined rapidly and easily by small portable instruments in the field, we concluded this empirical regression model could simulate both the seasonal and diurnal variations of P_n for *Miscanthus* leaves at different elevations.

Key words: chlorophyll content, C_4 plants, *Miscanthus*, photosynthesis, stomatal conductance.

不同海拔及季節下台灣原生芒屬植物之光合作用能力與葉綠素含量及氣孔導度間關係

翁仁憲 [1*] 翁韶良 [2] 陳忠偉 [3] 馮立德 [4]

1 中國醫藥大學　台中市學士路 91 號

2 行政院農業委員會特有生物研究保育中心　南投縣集集鎮民生東路 1 號

3 行政院農業委員會茶業改良場魚池分場　南投縣魚池鄉中山路 270 巷 13 號

4. 國立中興大學生命科學系　台中市國光路 250 號

*通訊作者：jhweng@mail.cmu.edu.tw

收件日期：2017 年 10 月 12 日；接受日期：2018 年 6 月 28 日

摘要

本研究於不同季節之高海拔(供試之物種爲：高山芒，*M. transmorrisonensis*，自然分布環境約海拔 3,000 公尺；臺灣芒，*M. sinenesis* var. *formosanus*，分布約 2,100 公尺)及低海拔(五節芒，*M. floridurus*，海拔約 70 公尺)地點測量不同葉綠素含量(*Chl*)之芒草葉片淨光合作用速率(P_n)。冬季高海拔山區氣溫(T)下降至攝氏 6 度時，高山芒葉片(葉綠素含量介於 0.17–0.46 g m^{-2})於測定光照強度 1,200 μmol m^{-2} s^{-1} 下測得之 P_n 值(P_{1200})仍可維持 0.4–3.2 μmol CO_2 m^{-2} s^{-1}。夏季氣溫回升，於攝氏 19 度下測量其 P_{1200} 值可達到 3.9–19.9 μmol CO_2 m^{-2} s^{-1}。低海拔地區之五節芒，於攝氏 25 或 30 度之氣溫條件下之 P_{1200} 及 P_{2000} (於光照強度 2,000 μmol m^{-2} s^{-1} 測得之 P_n 值)各可高達 30 及 40 μmol CO_2 m^{-2} s^{-1}。於相同測定溫度及光照強度之條件下，葉綠素含量較高之葉片會有較高的 P_n 及氣孔導度值(g_s)。然而，P_n–g_s 之決定係數(r^2)值較 P_n–*Chl* 之值高，且 P_n–g_s 之斜率變化與溫度有密切相關。當結合不同海拔、季節、光照強度下測量之資料後可得到顯著正相關之迴歸方程式($P_n = 1.147 + 4.222 \bullet g_s \bullet T$)，其推估之 P_n 值與實測之 P_n 值呈高度相關(回歸線爲幾近於 1:1 之直線，r^2=0.937, P<0.001)。更進一步以生育於海拔約 250 m 五節芒 ~~g_s~~ 之實測值(數據來自 Wong et al., 2014)驗證前揭迴歸方程式，推估之 P_n 值仍與實測值呈高度相關(r^2=0.856, P<0.001)。鑑於現場之 g_s 與 T 值可藉由可攜式小型儀器快速測得，本研究之芒草屬物種葉片可運用回歸方程式之模型模擬其於不同海拔高度下 P_n 值之季節及日變化。

關鍵詞：葉綠素含量、C4 型植物、芒草屬、光合作用、氣孔導度。

Introduction

Miscanthus is a genus of rhizomatous, perennial C_4 grass, which could be interbred with sugarcane to obtain new sugarcane cultivars (Chen *et al.* 1993). It recently has been used as a new energy or fiber crop in Europe and investigated for its biomass potential (Dohleman and Long 2009; Zub and Brancourt-Hulmel 2010) with the uniquely cold-tolerant ability (Beale *et al.* 1996; Naidu *et al.* 2003; Naidu and Long 2004; Wang *et al.* 2008) , and displays quite a good combination of radiation-, water- and nitrogen (N)-use efficiencies for biomass production (Dohleman and Long 2009; Zub and Brancourt-Hulmel 2010). Yet, the genus *Miscanthus* could achieve dry matter yield in excess of other C_4 crops in temperate climates (Beale *et al.* 1996; Dohleman and Long 2009).

Photosynthesis**,** one of the major determinants to biomass production and terrestrial carbon budgets, is influenced by many environmental and physiological factors (e.g. Berry and Downton 1982). C_4 plants are usually more sensitive to the low temperature than C_3 plants (Berry and Downton 1982; Sage and Kubien 2007), so there are few C_4 plants or non-existent in cooler climates (Berry and Downton 1982). However, *Miscanthus* is distributed from the tropical Pacific islands to northeastern Siberia, 50° N in the temperate zone (Clifton-Brown *et al.* 2008). In Taiwan (21°55’ N–25°18’ N) it grows from the coastline up to 3,200 m above sea level (a.s.l.), and forming a pure stand or the dominate species in early succession stage vegetation. According to the climate data of the Central Weather Bureau of Taiwan (1971–2000), mean temperatures of the warmest and coldest months of Alishan (23^o 31' N, 120^o 48' E, 2413 m a.s.l.) were 14.2 and 5.7 ^{o}C, respectively; while those of Taichung (24^o 09' N, 120^o 41' E, 84 m a.s.l.) were 28.5 and 16.2 ^{o}C, respectively. The atmospheric temperature decreases about 0.5 °C with the increases in elevation about 100 m (Weng *et al.* 2005). *Miscanthus* could maintain green leaves during the cold season from the low land to the sub-alpine zone in Taiwan. Their photosynthesis would face various temperature conditions at different elevations and in different seasons.

In the researches of *Miscanthus* × *giganteus*, it displayed a notable ability to maintain high photosynthetic rate at low temperatures of 14 ^{o}C or 10 ^{o}C (Beale *et al.* 1996; Naidu *et al.* 2003; Naidu and Long 2004; Farage *et al.* 2006; Wang *et al.* 2008; Dohleman *et al.* 2009), and remained ca. 1/5 of maximum photosynthetic rate (P) even at 5 ^{o}C (Naidu *et al.* 2003). We (Weng and Ueng 1997) and Kao *et al.* (1998) also found that, in Taiwan, *Miscanthus* showed the cold-tolerant abilities too; and the clones collected from high altitude showed better low-temperature adaptation in photosynthesis than those collected from low altitude. However, these studies were investigated in the laboratory at low elevation, and could not reflect the seasonal variation of photosynthesis at different elevation habitats.

For understanding the biomass production of vegetation grown at different habitats and conditions, it is necessary to monitor the spatial and temporal variations of photosynthetic capacity. However, the measurement of photosynthetic capacities in the field using traditional manners, such as gas analysis, is not an easy task due to the requirements of highly expensive equipment and intensive labor. In many species including C_4 plants and *Miscanthus*, the leaf chlorophyll content (*Chl*) and net photosynthetic rate (P_n) always closely related (Verhoeven *et al.* 1997; Weng and Hsu 2001; Zhao *et al.* 2005). Stomatal conductance (g_s) is another important limiting factor for photosynthesis. Not only *water or* moisture *deficits*, low-temperature and N-deficient stresses as well as low light intensity may also to enhance the closure of stomata (Beale *et al.* 1996; Dohleman *et al.* 2009; Wong, *et al.* 2014). Because *Chl* and stomatal conductance could be determined rapidly and easily by small portable instruments. Thus, the aim of the present study was to understand the photosynthetic capacity of *Miscanthus* leaves, with different *Chl* at high and low elevations of sub-tropical Taiwan through different seasons; and to elucidate the relationships of P_n to *Chl* and g_s.

Materials and methods

The top 2 fully expanded leaves of 3 *Miscanthus* species, with different *Chl*, were measured at 3 elevation sites in central Taiwan (Table 1). Among them, natural habitat-grown *M. transmorrisonensis* (natural distribution from 2,200 m to 3,100 m a.s.l.) and *M. sinensis* var. *formosanus* (1,000–2,400 m a.s.l.) were measured at 3,000 m a.s.l. (24^o 11’ N) site and at 2,100 m a.s.l. site (24^o 02’ N), respectively. While pot-grown *M. floridulus* (0–1,200 m a.s.l.) was measured on the campus of National Chung-Hsing University (24^o 09’ N, 70 m a.s.l.) (Table 1).

Table 1. Determination coefficient of regressions between net photosynthetic rate (P_n) and leaf chlorophyll content (*Chl*), and between P_n and stomatal conductance (g_s) of *Miscanthus* species at high and low elevations under different seasonal temperature and 1,200 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD).
表 1. 生長於高及低海拔之芒草屬供試物種，於不同季節之溫度下以 1,200 μmol m^{-2} s^{-1} 之光合作用光子通量密度(PPFD)測定所得淨光合作用速率(P_n)對葉綠素濃度(*Chl*)及對氣孔導度(g_s)之值經迴歸分析之決定係數。

Temperature (°C)	Determination coefficient (r^2)		Tested elevation (m)	Tested month	Tested species
	P_n-*Chl*	P_n-g_s			
6 (6.0)#	0.498**	0.003^{ns}	3,000	Dec.	*Miscanthus transmorrisonensis*
10 (8.9)	0.362*	0.019^{ns}	3,000	Mar.	
13 (10.7)	0.628^{*}	0.627^{*}	3,000	Apr.	
16 (11.8)	0.152^{ns}	0.730***	3,000	Nov.	
19 (15.8)	0.368*	0.829***	3,000	Jul.	
23 (19.8)	0.571**	0.892***	2,100	Jul.	*M. sinensis var. formosanus*
30 (28.7)	0.461*	0.888***	70	Jul.	*M. floridulus*
37 (28.6)	0.411*	0.985***	70	Aug.	

#The numeral inside and outside the parentheses is ambient temperature at 1000 h and leaf temperature throughout measurement, respectively. ***, **, * and ns: $P \le 0.001$, 0.01, 0.05 and > 0.05, respectively.
#括號內之數字表示測定當日 10 時 00 分之氣溫，括號外之數字表示現場進行測定時之葉片溫度。
***、**、*及 ns 分別表示迴歸分析 P 值不超過 0.001、0.01、0.05 及大於 0.05。

Sampling and measurements at 3,000 m and 2,100 m a.s.l. site

Due to *Chl* of natural habitat-grown *Miscanthus* may vary with soil properties, the *top 2 fully expanded* leaves with different *Chl* were select as materials. Leaves were sampled from a southeast-facing open stand. *Chl* was determined by *SPAD 502* chlorophyll meter (Minolta, Japan), and 12–16 leaves with different leaf color (*Chl* ranged about 0.1–0.5 g m^{-2}) were select for the measurements in each measuring period (Fig. 1). To calibrate the relationship between *Chl* and SPAD value, 3 fresh leaf disks (0.84 cm^2) from each leaf were extracted with 80% acetone and concentration of chlorophyll were determined by a spectrophotometer (U-2000, Hitachi, Japan) using the absorbance at 440.5, 645 and 663 nm by the equations of Arnon (1949).

All the leaf samples were collected before sunrise at 0530–0600 h and immediately recut under water, then the detached leaves were exposed to 600 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) by a halogen lamp, and maintained moist by spraying water until photosynthesis measurements were taken. Net photosynthetic rate (P_n) and stomatal conductance (g_s) were measured with a portable, open-flow gas exchange system (LI-6400, LI-COR Inc, USA), connected to a leaf chamber (6400-02B, LI-COR Inc, USA) and LED light source (6400-02, LI-COR Inc, USA). Measurements were made during 1000–1500 h under 1,200 μmol m^{-2} s^{-1} PPFD, and the leaf temperature (T) was maintained constant, ca. 2 °C higher than ambient temperature at 1000 h which was 6 °C (Dec.), 10 °C (Mar.), 13 °C (Apr.), 16 °C (Nov.) and 19 °C (Jul.) at 3,000 m a.s.l. site, and 23 °C (Jul.) at 2,100 m a.s.l. site.(Fig. 1).

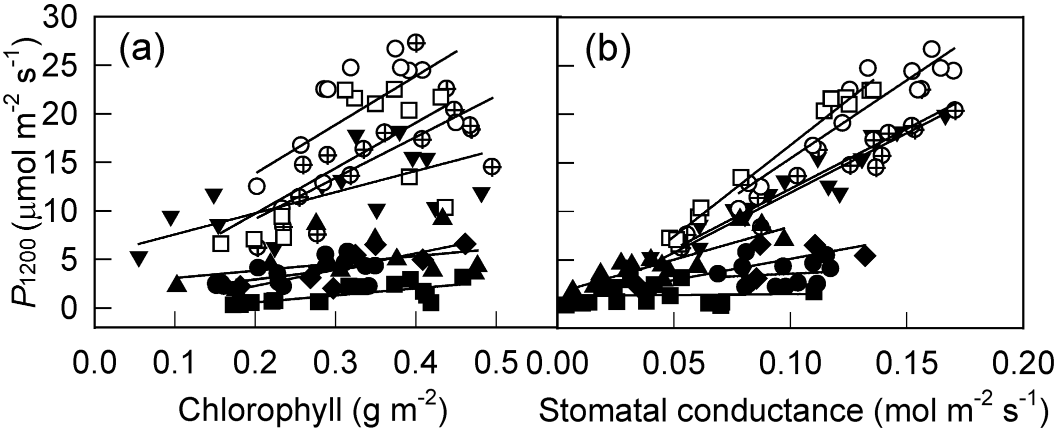

Fig. 1. The relationships of net photosynthetic rate to chlorophyll content and to stomatal conductance of *Miscanthus* leaves measured under 1,200 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and different leaf temperature. ■: 6 °C, ●: 10 °C, ◆: 13 °C, ▲: 16 °C and ▼: 19 °C at 3,000 m site; ⊕: 23 °C at 2,100 m site; and ○: 30 °C and □: 37 °C at 70 m site.

圖 1. 供試之芒草屬葉片於不同葉片溫度下以 1,200 μmol m^{-2} s^{-1} 之光合作用光子通量密度(PPFD)測定所得淨光合作用速率(P_n)對葉綠素濃度(*Chl*)及對氣孔導度(g_s)之變化。測量地點海拔 3,000 公尺，■: 6 °C、●: 10 °C、◆: 13 °C、▲: 16 °C 及▼: 19 °C；海拔 2,100 公尺處，⊕: 23 °C；海拔 70 公尺處，○: 30 °C 及□: 37 °C。

Cultivation and measurements at 70 m a.s.l. site

M. floridulus collected from lowlands in central Taiwan was potted (38 cm-diameter) in a mix of soil : vermiculite : sand = 1:1:1, and placed outdoors to receive regular water and full sunlight on the campus of National Chung-Hsing University (24^{o} 10’ N, 70 m a.s.l.), Taichung, Taiwan. In order to obtain leaf samples with different *Chl*, 0, 1 and 2 g N fertilizer per pot were applied at 4 and 2 weeks before measurement, respectively. The P_n and g_s were measured by two methods. The first method was similar to measured at high elevation sites, i.e. P_n and g_s of 12 attached leaves were measured under 1,200 μmol m^{-2} s^{-1} PPFD, and maintained T at 30°C (Jul.) and 37 °C (Aug.). The second method was P_n of 3–5 attached leaves with different *Chl* was measured under 4–5 levels of PPFD, and maintained T at 20 °C (Jan.), 25 °C (Mar.) and 30 °C (Jul,), respectively (Fig. 2).

Initially, the leaves were exposed to 800 or 1,200 μmol m^{-2} s^{-1} PPFD, then PPFD was raised to 2,000 mol m^{-2} s^{-1}, and stepwise from high to low levels of PPFD, i.e., 800, 400 and 200 μmol m^{-2} s^{-1}. Under each level of PPFD, P_n and g_s were taken every 5 min until it reaches to steady. The same equipment as measured at high elevation sites was used in both methods.

Results

At the same *T* and PPFD, the leaves with higher *Chl* always had higher P_n and g_s (Figs. 1 and 2). For the measurements under 1,200 μmol m^{-2} s^{-1} PPFD, the slopes of the regression lines to P_{1200} and P_n–*Chl* and P_{1200}–g_s were increased with the increase of *T* (Figs. 1 and 3), and the latter correlation always showed higher determination coefficient (r^2) than the former, especially at high *T* (Table 1). Even at 6 °C, *M. transmorrisonensis* leaves (*Chl* ranged 0.17–0.46 g m^{-2}) could retain 0.4–3.2 μmol CO$_2$ m^{-2} s^{-1} of P_{1200}. While in the summer, P_{1200} could raise to 3.9–19.9 μmol CO$_2$ m^{-2} s^{-1}, when measured at 19 °C. At low elevation, *M. floridulus* could raise its P_{1200} and P_{2000} (P_n measured under 2,000 μmol m^{-2} s^{-1} PPFD) to about 30 and 40 μmol CO$_2$ m^{-2} s^{-1}, at both 25 and 30 °C, respectively (Figs. 1 and 3).

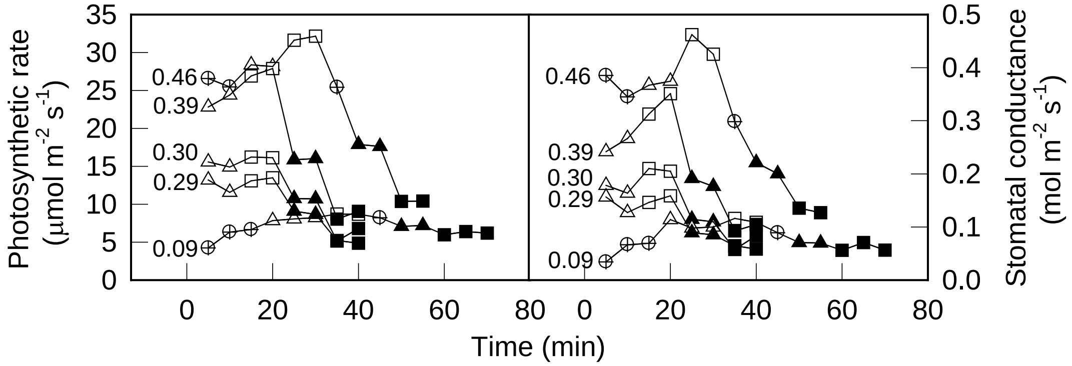

Fig. 2. The variation of net photosynthetic rate and stomatal conductance of 5 *Miscanthus floridulus* leaves with different chlorophyll content, under 20 °C and variant photosynthetic photon flux density (PPFD) (■: 200, ▲: 400 ~~℃~~, ⊕: 800, △: 1,200 and □: 2,000 μmol m^{-2} s^{-1}). Each line represents the results of a leaf, numeral before each line is the chlorophyll content (g m^{-2}) of a tested leaf.

圖 2. 不同葉綠素濃度之五節芒(*Miscanthus floridulus*)葉片 5 片於固定攝氏 20 度，不同光合作用光子通量密度(PPFD)(■: 200, ▲: 400, ⊕: 800, △: 1,200 and □: 2,000 μmol m^{-2} s^{-1})下淨光合作用速率(P_n)及氣孔導度(g_s)之變化。圖所示之折線表示 1 片供試葉片之數據，折線起點旁之數字表示該葉片之葉綠素濃度(g m^{-2})。

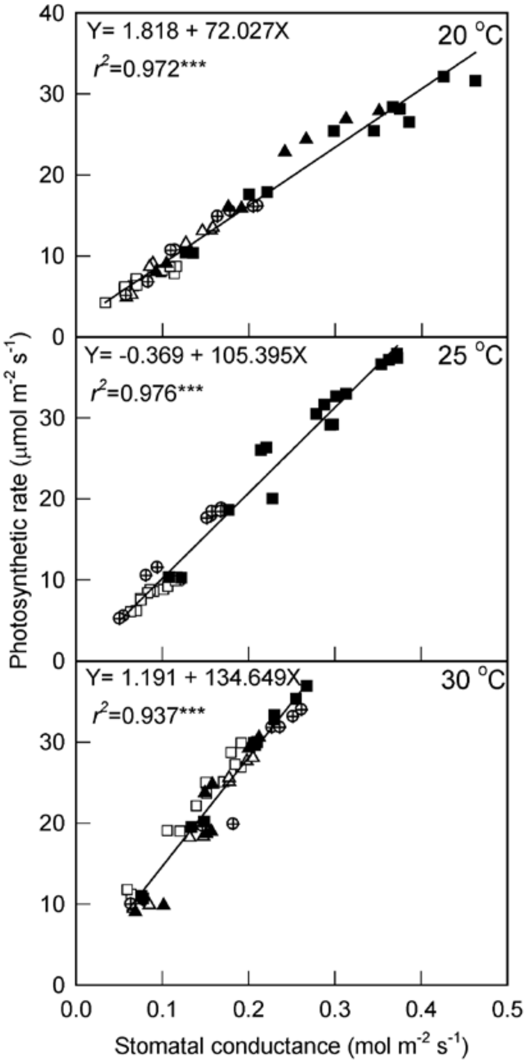

Fig. 3. Relationship between net photosynthetic rate and stomatal conductance of *Miscanthus floridulus* leaves with different chlorophyll content, measured under variant photosynthetic photon flux density (PPFD) and different leaf temperature. The same symbol in each panel represents the same leaf measured under different photosynthetic photon flux density. ***: P<0.001. n=3–5

圖 3. 五節芒(*Miscanthus floridulus*)不同葉綠素濃度之葉片，於不同的光合作用光子通量密度(PPFD)及溫度下淨光合作用速率(P_n)及氣孔導度(g_s)之關係。各小圖內相同的符號表示同一葉片於不同PPFD 下所測得之資料。***：P<0.001。n=3–5。

For the variation of P_n and g_s under varied PPFD, measured at 20 °C was selected as example shows in Fig. 2. Both P_n and g_s varied closely with the variation of PPFD, and the leaves with higher *Chl* always showed higher P_n and g_s under ~~a~~ the same level of PPFD. Even data obtained from leaves with different *Chl* and at different PPFD were pooled together for analysis, P_n was closely related to g_s. Yet, the slope of the regression equation to P_n–g_s was increasing with T increased (Fig. 3). Fig. 4 also shows that, when merging data measured at different elevation and season, the slopes of the regression equations to P_n–g_s still showed very high positive linear correlation with T.

Due to P_n–g_s relationship always showed higher r^2 than P_n–*Chl* relationship (Table 1), and the slopes of P_n–g_s relationships closely related to T (Fig. 4). For obtaining the "best-fit" combination of P_n with g_s and T, we combined these two relationships to analysis. In other words, the resulting regression equation to P_n and g_s could be fitted as equation (1) (Figs. 1b, 3 and Table 1), and the regression equation to slope (b) and T could be fitted as equation (2) (Fig. 4). Subsequently, equation (3) was obtained by combining equations (1) to (2).

$$P_n = a + b \bullet g_s \tag{1}$$

$$b = c + d \bullet T \tag{2}$$

$$P_n = a + c \bullet g_s + d \bullet g_s \bullet T \tag{3}$$

$$P_n = 1.147 + 4.222 \bullet g_s \bullet T \tag{4}$$

Based on multiple regression analysis, the regression coefficient of equation (3) could be further fitted (a=1.919, c= –35.592 and d=5.474). It showed high determination coefficient (r^2=0.950, P<0.001), with partial correlation coefficients of g_s and $g_s \bullet T$ to P_n were –0.308 (20%) and 1.255 (80%), respectively. Due to c showed negative value and low partial correlation coefficients to P_n (20%), thus, we used $P_n = a + d \bullet g_s \cdot T$ to fit the relationship between P_n and g_s at varied level of T and PPFD. Obtained result [equation (4)] indicated that P_n showed a very high positive linear correlation with $g_s \bullet T$ (r^2=0.937, P<0.001, Fig. 5). Fig. 6 shows that P_n estimated from equation (4) was closely related to measured P_n (regression line approximate to 1:1 line, r^2=0.937, P<0.001). This relationship existed when data were obtained by using leaves with yellow-green to dark-green colors of 3 *Miscanthus* species grown at high and low elevations, and measured at 1,200 or 200–2,000 μmol m^{-2} s^{-1} PPFD through different season.

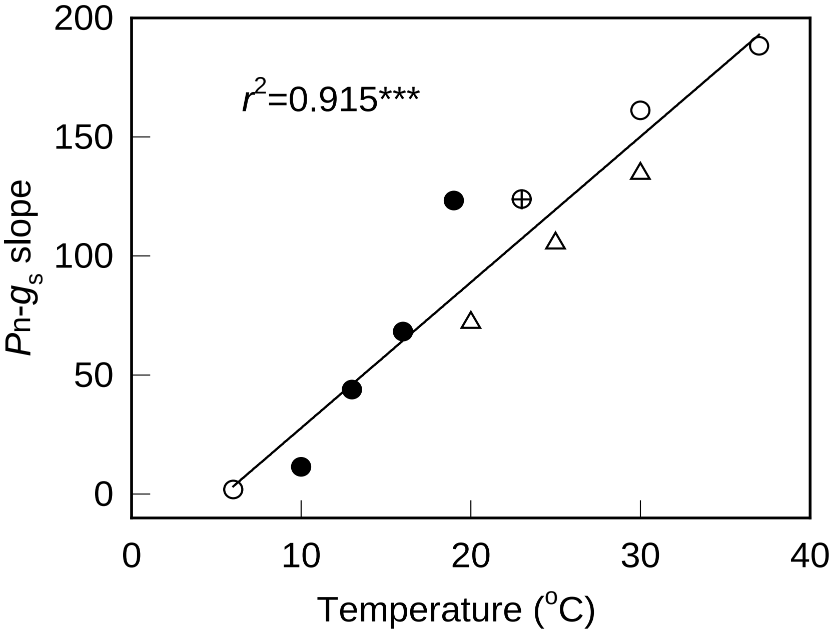

Fig. 4. The relationship of the slope of the regression between net photosynthetic rate and leaf stomatal conductance (P_n-g_s slope) to leaf temperature of *Miscanthus* leaves.

●, ⊕ and ○: Measured under 1,200 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at 3,000 , 2,100 and 70 m a.s.l. site, respectively (data from Fig. 1b). △: Measured under variant PPFD at 70 m a.s.l. site (data from Fig. 3). ***: P<0.001

圖 4. 供試芒草葉片之淨光合作用速率與氣孔導度資料經迴歸分析後所得 P_n-g_s 斜率對葉片溫度之變化。●、⊕及○：符號依序分別於海拔 3,000、2,100 及 70 公尺之地點，固定光合作用光子通量密度(PPFD) 1,200 μmol m^{-2} s^{-1} 條件下測量之數據(資料來自圖 1b)。△：於海拔 70 公尺之地點，不同的 PPFD 條件下測得之數據(資料來自圖 3)。***： P<0.001。

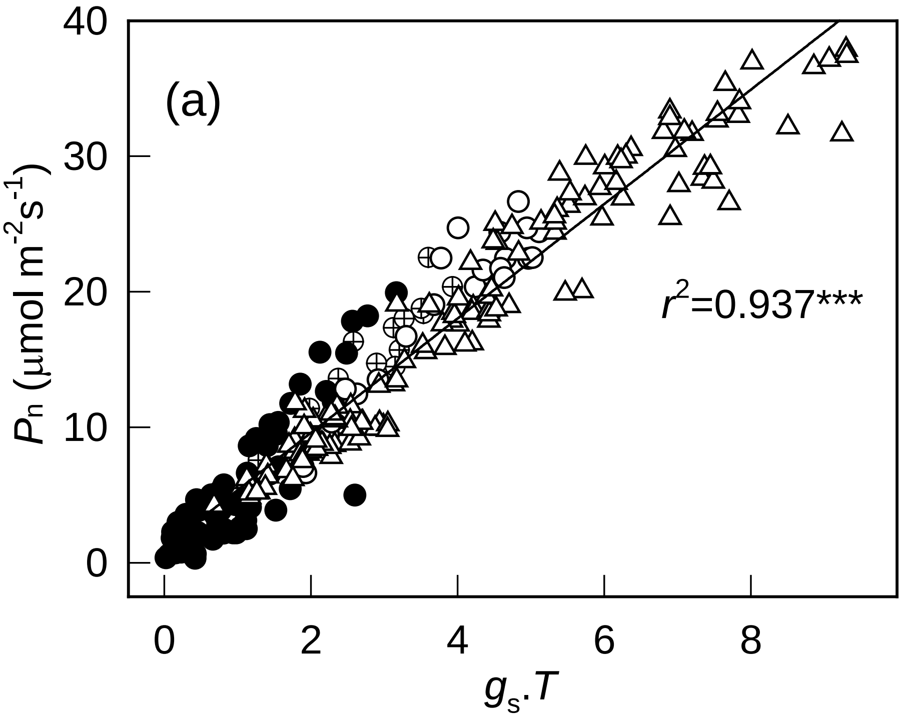

Fig. 5. Relationship between net photosynthesis rate (P_n) and the product of leaf stomatal conductance and leaf temperature (g_s•T). ●, ⊕ and ○: Measured under 1,200μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at 3,000 , 2,100 and 70 m a.s.l. site, respectively (data from Fig. 1b). △: Measured under varietal PPFD and temperature at 70 m a.s.l. site (data from Fig. 3). ***: P<0.001.

圖 5. 淨光合作用速率(P_n)與葉片氣孔導度及溫度之乘積(g_s•T)之間的關係。●、⊕及○：符號依序分別於海拔 3,000、2,100 及 70 公尺之地點，固定光合作用光子通量密度(PPFD) 1,200 μmol m^{-2} s^{-1} 條件下測量之數據(資料來自圖 1b)。△：於海拔 70 公尺之地點，不同的 PPFD 條件下測得之數據(資料來自圖 3)。***： P<0.001。

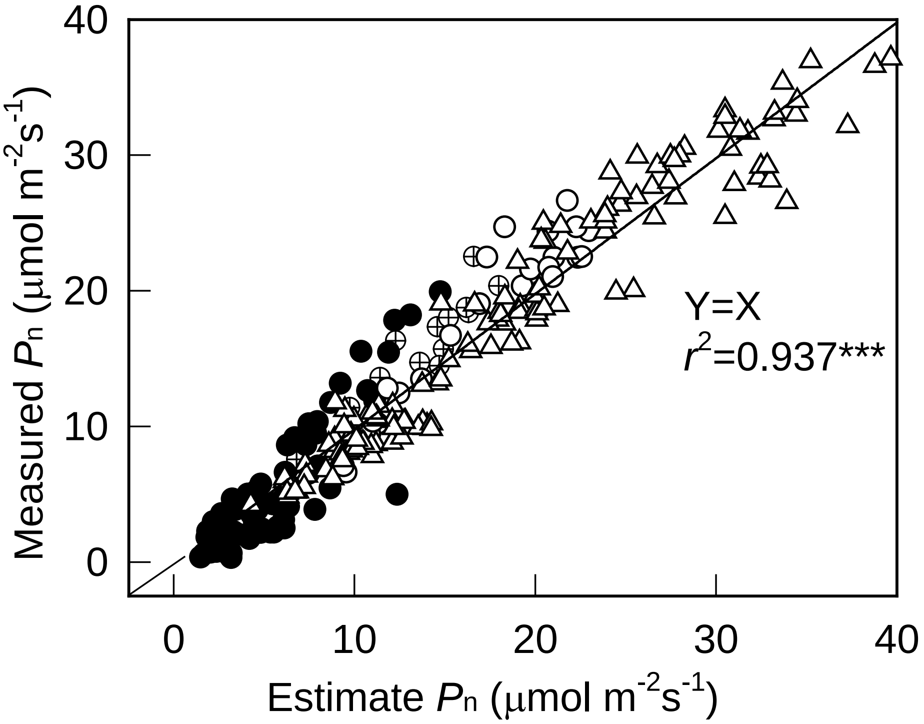

Fig. 6. Relationship between the measured and estimated net photosynthetic rate (P_n), from the equation of P_n =1.147+4.222• g_s•T. g_s and T: Stomatal conductance and leaf temperature, respectively. ●, ⊕ and ○: Measured under 1,200μmol m^{-2} s^{-1} photosynthetic photon flux density at 3,000 , 2,100 and 70 m a.s.l. site, respectively (data from Fig. 1b). △: Measured under 20–30 °C leaf temperature and 800–2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density at 70 m a.s.l. site (data from Fig. 3). ***: P<0.001.

圖 6. 實測淨光合速率與迴歸方程式(P_n =1.147+4.222• g_s•T)推估淨光合作用速率之關係。g_s及 T：分別爲氣孔導度及葉片溫度。●、⊕及○：符號依序分別於海拔 3,000、2,100 及 70 公尺之地點，固定光合作用光子通量密度(PPFD) 1,200 μmol m^{-2} s^{-1} 條件下測量之數據(資料來自圖 1b)。△：於海拔 70 公尺之地點，葉片溫度 20–30 °C 及 800–2,000 μmol m^{-2} s^{-1} PPFD 之條件下測得之數據(資料來自圖 3)。***： P<0.001。

Discussion

Unlike the lowlands at high latitudes, tropical alpine shows lower seasonal variation of temperature (Smith and Young, 1987). As a result that the summer is cooler and the winter is warmer than in lowlands of temperate region. At 3,000 m a.s.l. site, the monthly mean air temperature of the coldest month (Jan.) is ca. 2 °C, thus, leaves of *Miscanthus* often subject to freezing temperature. Nevertheless, *M. transmorrisonensis* could maintain a lot of activity green leaves through winter at here (Table 1 and Fig. 1), indicating that *M. transmorrisonensis* is uniquely cold-tolerant among C_4 plants.

Pigments in leaves are integrally related to photosynthesis, and can be non-destructively estimated through optical methods. Among them, chlorophyll was considered as a short-term indicator for photosynthetic capacity (Zhao *et al.*, 2005). The results of the present study showed that the leaves with higher *Chl* always had higher P_n, when compared at the same T and PPFD (Figs 1 and 2). These results were consistent with the previous papers (Weng and Hsu, 2001; Zhao *et al.*, 2005; Wong *et al.*, 2014). However, as the presents in Table 1, r^2 of P_{1200}–*Chl* regressions always lower than those of the P_{1200}–g_s regressions. This result might be due to *Chl* fail to detect dynamic variations of P_n like those occurring during the day or under certain stress conditions (Nichol *et al.*, 2000; Evain *et al.*, 2004).

Previously, we compared the photosynthetic capacity of 17 *Miscanthus* clones at 3 levels of N application. Results indicated that the saturated P_n was closely related to leaf conductance, even data from all tested clones and N levels were merged to analysis (Weng and Hsu, 2001). Moreover, decreased leaf P_n due to N deficiency was mainly associated with lower g_s rather than carboxylation capacity of leaf chemistry (Zhao *et al.*, 2005). The physiological role of stomata is to prevent water loss and to facilitate CO_2 diffusion to mesophyll cells. To optimize the water use efficiency, guard cells could monitor the plant water status and the CO_2 demand from the mesophyll. Reports have been point out that guard cells respond to the intercellular CO_2 concentration, which is determined by atmospheric CO_2 and by the mesophyll assimilation rate (Vavasseur and Raghavendra, 2005), allowing a tight coupling between g_s and P in many conditions, included changing light intensity (D’ambrosio *et al.*, 2003; Huxman and Monson, 2003), temperature (Pittermann and Sage, 2000, 2001), leaf N status (Weng and Hsu, 2001; Mohotti and Lawlor, 2002) as well as changing conditions due to the diurnal (Hirasawa and Hsiao, 1999; Mohotti and Lawlor, 2002, Cuevas *et al.*, 2006; Ding *et al.*, 2006; Leakey *et al.*, 2006), seasonal (Hirasawa and Hsiao, 1999; Leakey *et al.*, 2006) and genotypical (Ding *et al.*, 2006; Dohleman *et al.*, 2009; Dohleman and Long, 2009) variations.

In the present study, we found that the slope of P_n–g_s regression was increasing with T increased (Figs. 1b and 3). What is the reason for this phenomenon? Reports have pointed out that both g_s and P always decreased with decreasing temperature. In many C_4 plants, the decreasing amplitude of P following the temperature decrease was higher than that of g_s (Pittermann and Sage, 2000, 2001; Kubien *et al.*, 2003; Kościelniak and Biesaga-Kościelniak, 2006). As a result, the sensitivity of P_n to variation in g_s generally decreases at colder temperatures, and this was due to the biochemical controls over P_n at low temperature was less sensitive to changes in intercellular CO_2 concentration. Thus, temperature change may disproportionately alter g_s relative to P_n, and stomatal limitations to C_4 photosynthesis are generally smaller at low temperature (Sage and Kubien, 2007). This reason may lead to the decline of slope for P_n–g_s regression line (Figs. 1b, 3 and 4).

We combined the P_n–g_s relationships under different T, and found that P_n showed a significant positive regression with gs•T. Based on the close correlation (regression line approximate to 1:1 line, r^2=0.937, P<0.001) between the P_n estimated from the single regression equation (4) and the P_n measured in field, when data obtained from different illumination and seasonal temperature conditions were pooled together for statistical analysis (Fig. 6). Moreover, we used another published data (Wong et al., 2014), with a broad range of P_n caused by different light, humidity, seasonal temperature, phenological stage and N fertilization, to estimated P_n from equation (4). The results showed that the estimated P_n still correlated linearly with the measured P_n (r^2=0.856, P<0.001, Figure omitted). We, thus, concluded this empirical regression model could simulate both the seasonal and diurnal variations of P_n for *Miscanthus* leaves grown at high and low elevations with different *Chl*. Because g_s and T could be determined rapidly and easily by small portable instruments in the field, this empirical regression model might be of benefit in eco-physiological research and monitoring.

References

Arnon, D.J. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1-15.

Beale, C.V., D.A. Bint and S.P. Long. 1996. Leaf photosynthesis in the C_4-grass *Miscanthus* × *giganteus,* growing in the cool temperate climate of southern England. Journal of Experimental Botany 47: 267-273.

Berry, J.A. and W.J.S. Downton. 1982. Environmental regulation of photosynthesis. pp. 263-343. *In*: Govindjee (ed.). Photosynthesis Volume II. Academic Press, London, United Kingdom.

Chen, Y.H., C. Chen and C.C. Lo. 1993. Studies on anatomy and morphology in *Saccharum-Miscanthus* nobilized hybrids. I. Transmission of tilling, ratooning, adaptation and disease resistance from *Miscanthus* spp. Journal of the Agricultural Association of China 164: 31-45.

Clifton-Brown, J., Y.C. Chiang and T.R. Hodkinson. 2008. *Miscanthus*: Genetic Resources and Breeding Potential to

Enhance Bioenergy Production. pp. 273-294 *In*: W. Vermerris (ed.). Genetic Improvement of Bioenergy Crops. Springer-Verlag, New York, United States of America.

Cuevas, E., P. Baeza and J.R. Lissarrague. 2006. Variation in stomatal behaviour and gas exchange between mid-morning and mid-afternoon of north–south oriented grapevines (*Vitis vinifera* L. cv. Tempranillo) at different levels of soil water availability. Scientia Horticulturae 108: 173-180.

D'Ambrosio, N., Arena, C., Virzo de Santo, A., 2003. Different relationship between electron transport and CO_2 assimilation in two *Zea mays* cultivars as influenced by increasing irradiance. Photosynthetica 41, 489–495.

Ding, L., K.J. Wang, G.M. Jiang, Y.G. Li, C.D. Jiang, M.Z. Liu, S.L. Niu and Y. Peng. 2006. Diurnal variation of gas exchange, chlorophyll fluorescence, and xanthophyll cycle components of maize hybrids released in different years. Photosynthetica 44: 26-31.

Dohleman, F.G., E.A. Heaton, A.D.B. Leakey and S.P. Long. 2009. Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of *Miscanthus* relative to switchgrass? Plant, Cell & Environment 32: 1525-1537.

Dohleman, F.G. and S.P. Long. 2009. More productive than maize in the midwest: How does *Miscanthus* do it? Plant Physiology 150: 2104-2115.

Evain, S., J. Flexas and I. Moya. 2004. A new instrument for passive remote sensing: 2. Measurement of leaf and canopy reflectance changes at 531 nm and their relationship with photosynthesis and chlorophyll fluorescence. Remote Sensing of Environment 91: 175-185.

Farage, P.K., D. Blowers, S.P. Long and N.R. Baker. 2006. Low growth temperatures modify the efficiency of light use by photosystem II for CO_2 assimilation in leaves of two chilling-tolerant C_4 species, *Cyperus longus* L. and *Miscanthus* × *giganteus*. Plant, Cell & Environment 29: 720-728.

Hirasawa, T. and T.C. Hsiao. 1999. Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. Field Crops Research 62: 53-62.

Huxman, T.E. and R.K. Monson. 2003. Stomatal responses of C_3, C_3-C_4 and C_4 *Flaveria* species to light and intercellular CO_2 concentration: implications for the evolution of stomatal behaviour. Plant, Cell & Environment 26: 313-322.

Kao, W.Y., T.T. Tsai and W.H. Chen. 1998. A comparative study of *Miscanthus floridulus* (Labill) Warb and *M. transmorrisonensis* Hayata: Photosynthetic gas exchange, leaf characteristics and growth in controlled environments. *Annals of Botany* 81: 295-299.

Kościelniak, J. and J. Biesaga-Kościelniak. 2006. Photosynthesis and non- photochemical excitation quenching components of chlorophyll excitation in maize and field bean during chilling at different photon flux density. Photosynthetica 44: 174-180.

Kubien, D.S., S. von Caemmerer, T. Robert, R.T. Furbank and R.F. Sage. 2003. C_4 photosynthesis at low temperature. A study using transgenic plants with reduced amounts of Rubisco. Plant Physiology 132: 1577-1585.

Leakey, A.D.B., M. Uribelarrea, E.A. Ainsworth, S.L. Naidu, A. Rogers, D.R. Ort and S.P. Long. 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiology 140: 779-790.

Mohotti, A.J. and D.W. Lawlor. 2002. Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. Journal of Experimental Botany 53: 313-322.

Naidu, S.L. and S.P. Long. 2004. Potential mechanisms of low-temperature tolerance of C_4 photosynthesis in *Miscanthus*·× *giganteus*: an in vivo analysis. Planta 220: 145-155.

Naidu, S.L., S.P. Moose, A.K. AL-Shoaibi, C.A. Raines and S.P. Long. 2003. Cold tolerance of C_4 photosynthesis in *Miscanthus*·× *giganteus*: Adaptation in amounts and sequence of C_4 photosynthetic enzymes. Plant Physiology 132: 1688-1697.

Nichol, C.J., K.F. Huemmrich, T.A. Black, P.G. Jarvis, C.L. Walthall, J. Grace and F.G. Hall. 2000. Remote sensing of photosynthetic light-use efficiency of boreal forest. Agricultural and Forest Meteorology 101: 131-142.

Pittermann, J. and R.F. Sage. 2000. Photosynthetic performance at low temperature of *Bouteloua gracilis* Lag., a high-altitude C_4 grass from the Rocky Mountains, USA. Plant, Cell & Environment 23: 811-823.

Pittermann, J. and R.F. Sage. 2001. The response of the high altitude C_4 grass *Muhlenbergia montana* (Nutt.) A.S. Hitchc. to long-and short-term chilling. Journal of Experimental Botany 52: 829-838.

Sage, R.F. and D.S. Kubien. 2007. The temperature response of C_3 and C_4 hotosynthesis. Plant, Cell & Environment 30: 1086-1106.

Smith, A.P. and T.P. Young. 1987. Tropical alpine plant ecology. Annual Review of Ecology and Systematics 18: 137-158.

Vavasseur, A. and A.S. Raghavendra. 2005. Guard cell metabolism and CO_2 sensing. New Phytologist 165: 665-682.

Verhoeven, A.S., B. Demmig-Adams and W.W. Adams III. 1997. Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. Plant Physiology 113: 817-824.

Wang, D., A.R.Jr. Portis, S.P. Moose and S.P. Long. 2008. Cool C_4 photosynthesis: pyruvate Pi dikinase expression and activity corresponds to the exceptional cold tolerance of carbon assimilation in *Miscanthus*·× *giganteus*. Plant Physiology 148: 557-567.

Weng, J.H. and F.H. Hsu. 2001. Gas exchange and epidermal characteristics of *Miscanthus* populations in Taiwan varying with habitats and nitrogen application. Photosynthetica 39: 35-41.

Weng, J.H., T.S. Liao, K.H. Sun, J.C. Chung, C.P. Lin and C.H. Chu. 2005. Seasonal variation in photosynthesis of *Picea morrisonicola* grown in the subalpine region of subtropical Taiwan. Tree Physiology 25: 973-979.

Weng J.H. and R.G. Ueng. 1997. Effect of temperature on photosynthesis of *Miscanthus* clones collected from different elevations. Photosynthetica 34: 307-311.

Wong, S. L., C.W. Chen, M.Y. Huang and J.H. Weng. 2014. Relationship between photosynthetic CO_2 uptake rate and electron transport rate in two C_4 perrenial grasses under different nitrogen fertilization, light and temperature conditions. Acta Physiologiae Plantarum 36: 849-857.

Zhao, D., K.R. Reddy, V.G. Kakani and V.R. Reddy. 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. European Journal of Agronomy 22: 391-403.

Zub, H.W. and M. Brancourt-Hulmel. 2010. Agronomic and physiological performances of different species of *Miscanthus*, a major energy crop. A review. Agronomy for Sustainable Development 30: 201-214.