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Using combined measurements of gas exchange and chlorophyll fluorescence to investigate the photosynthetic light responses of plant species adapted to different light regimes

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Abstract

One broad-leaved pioneer tree, *Alnus formosana*, two broad-leaved understory shrubs, *Ardisia crenata* and *Ardisia cornudentata*, and four ferns with different light adaptation capabilities (ranked from high to low, *Pyrrosia lingus*, *Asplenium antiquum*, *Diplazium donianum*, *Archangiopteris somai*) were used to elucidate the light responses of photosynthetic rate and electron transport rate (ETR). Pot-grown materials received up to 3 levels of light intensity, *i.e.*, 100%, 50% and 10% sunlight. Both gas exchange and chlorophyll (Chl) fluorescence were measured simultaneously by an equipment under constant temperature and 7 levels (0–2,000 μmol m^{-2} s^{-1}) of photosynthetic photon flux density (PPFD). Plants adapted to- or acclimated to high light always had higher light-saturation point and maximal photosynthetic rate. Even materials had a broad range of photosynthetic capacity [maximal photosynthetic rate ranging from 2 to 23 μmol(CO_2) m^{-2} s^{-1}], the ratio of ETR to gross photosynthetic rate (P_G) was close for *A. formosana* and the 4 fern species when measured under constant temperature, but the PPFD varied. In addition, *P. lingus* and *A. formosana* grown under 100% sunlight and measured at different seasonal temperatures (15, 20, 25, and 30°C) showed increased ETR/P_G ratio with increasing temperature and could be fitted by first- and second-order equations, respectively. With this equation, estimated and measured P_G were closely correlated (r^2 = 0.916 and r^2 = 0.964 for *P. lingus* and *A. formosana*, respectively, $p<0.001$). These equations contain only the 2 easily obtained dynamic indicators, ETR and leaf temperature. Therefore, for some species with near ETR/P_G ratio in differential levels of PPFD, these equations could be used to simulate dynamic variation of leaf scale photosynthetic rate under different temperature and PPFD conditions.

Additional key words: electron transport rate; fern; gross photosynthetic rate; light intensity; temperature; woody plant.

Introduction

Sunlight is the energy source for plant photosynthesis and one of the major environmental factors influencing growth and distribution of plant species (Boardman 1977, Lambers *et al.* 1998). Studying photosynthesis-irradiance relationships is fundamental to plant ecophysiological research and rare species management, because the photosynthetic light-response curve could be used to assess the light-utilizing ability and optimal habitat light condition of plants (Bazzaz and Carlson 1982, Lüttge 1997, Aleric and Kirkman 2005).

Plant species adapted to different light regimes show differential photosynthetic characteristics. Species adapted to high light often have a high light-compensation point and light-saturation point and maximal photosynthetic rate (Givnish 1988, Hölscher *et al.* 2006). In addition, the same plant species grown under different light environments should be able to acclimate to the habitat by morphological and physiological changes (Griffin *et al.* 2004, Aleric and Kirkman 2005, Huang *et al.* 2007, Zhang *et al.* 2007, Dai *et al.* 2009). However, the capability of photosynthetic adaptation and acclimation is species-specific. For example, in sun plants, the maximal rate of photosynthesis for sun leaves is always greater than for shade leaves (Pearcy and Sims 1994). In contrast,

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Abbreviations: Chl – chlorophyll; ETR – electron transport rate; F_v/F_m – potential quantum efficiency of PSII; g_s – stomatal conductance; P_G – gross photosynthetic rate; P_N – net photosynthetic CO_2-exchange rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; Φ_{PSII} – PSII efficiency.

this rate for medium-light-grown individuals of species adapted to the shade (Chabot and Chabot 1977, Dai *et al.* 2009) or a broad light range (Aleric and Kirkman 2005, Zhang *et al.* 2007) is generally higher than that for high- and low-light-grown plants. Yet, some shade species have a similar rate of photosynthesis if grown in the shade or full sun (Griffin *et al.* 2004).

Photosynthesis is a combination of light and carbon reactions. The former converts light energy to the generation of ATP and NADPH, which are consumed by the carbon reaction (Blankenship 2006). Insufficient light may limit photosynthesis, thus resulting in reduced net carbon gain and plant growth. In contrast, under high irradiance, *e.g.*, leaves in the top canopy layer exposed to sun, or leaves in the shade exposed to sunflecks, the light reaction may absorb more photons than the dark reaction can use, and this excessively absorbed energy often leads to reduced efficiency of the photosystem, especially photosystem II (PSII) (Demmig-Adams *et al.* 1996, Kato *et al.* 2003, Adams *et al.* 2004). Plants have developed a number of strategies to balance the captured photon energy and protect the photosynthetic apparatus against photodamage. Among them, xanthophylls-dependent non-photochemical quenching can play an important role to dissipate the excess energy as heat (Demmig-Adams and Adams 1996, Li *et al.* 2000, Adams *et al.* 2004). Thus, the light energy absorbed by the photosystem can be consumed by photochemical and nonphotochemical processes. With increasing light intensity, the proportion of excess light energy increases. Therefore, under high light, often the xanthophyll cycle is enhanced and PSII efficiency decreased. Nevertheless, the xanthophyll cycle and PSII efficiency gradually recover when light becomes weak (Demmig-Adams *et al.* 1996, Verhoeven *et al.* 1999). As well, PSII efficiency might decrease with decreased photosynthetic rate, when photosynthesis is inhibited by environmental or physiological factors (Ghannoum *et al.* 2003, Adams *et al.* 2004, Weng 2009). Stomatal conductance (g_s) is another important limiting factor for photosynthesis. Photosynthesis and g_s may be tightly coupled in many conditions involving at different light intensity and temperature (Yu *et al.* 2004, Huang *et al.* 2007).

The traditional measurement of photosynthetic rate, especially in the field, is not easy because it requires expensive equipment and intensive labor. Nevertheless, Chl fluorescence quenching analysis is a fast, simple, noninvasive, and reliable method to assess changes in function of PSII under different environmental and physiological conditions (Roháček and Barták 1999, Maxwell and Johnson 2000). ETR, calculated from the product of PSII efficiency and absorbed light, expresses the relative rate of electron transport through PSII (Krall and Edwards 1992). Because photosynthetic CO_2 fixation is a major sink for electrons from PSII, ETR is related to the rate of photosynthesis (*e.g.*, Krall and Edwards 1992), with similar patterns between the light-response curve of ETR and CO_2 fixation (Earl and Tollenaar 1998, Franco and Lüttge 2002, Coopman *et al.* 2008). However, except for CO_2 fixation, electrons from PSII have several energy sinks, such as photorespiration (Peterson 1994), water-water cycle (Asada 1999) and the cyclic electron flow within PSII (Miyake and Okamura 2003), as well as nitrogen assimilation (Robinson 1990). Because alternative electron paths, mostly photorespiration, are restricted in C_4 plans, numerous studies have concluded a strong linear relationship between ETR and the gross photosynthetic rate (P_G). This relationship appears to be stable under many conditions, even when merging data from different genotypes (Earl and Tollenaar 1998) or from differences in both CO_2 partial pressure and temperature (Kakani *et al.* 2008) across a broad range of light intensity.

In C_3 plants, both CO_2 fixation and photorespiration are major sinks for electrons from PSII. Therefore, the ratio of ETR to P_G [or PSII efficiency (Φ_{PSII})/photosynthetic rate per absorbed quantum (Φ_{CO2})] greatly increases with decreasing CO_2 partial pressure (Krall and Edwards 1990, Cornic and Briantais 1991) or increasing temperature (Oberhuber and Edwards 1993) and O_2 partial pressure (Ripley *et al.* 2007) because of the increase in photorespiration. Thus, in C_3 plants, ETR and photosynthetic rate are significantly correlated only under conditions of nonphotorespiratory or with approximate temperature as well as CO_2 and O_2 concentrations (Cheng *et al.* 2001, Pérez-Torres *et al.* 2007, Ripley *et al.* 2007).

From the previously mentioned reports, photosynthetic light response varies by plant species, and Chl fluorescence measurement is a simple and reliable method for estimating photosynthesis. Ecophysiological studies require knowledge of photosynthetic rate of plants under different environments and with a broad range of light intensity. However, few studies of species with different taxa and capabilities of light adaptation and acclimation have compared the light-response patterns of ETR and CO_2 fixation of C_3 plants (Franco and Lüttge 2002, Sun *et al.* 2007). In this study, we used 3 woody and 4 fern species with different light-adaptation capabilities to elucidate the light response patterns of ETR and CO_2 fixation.

Materials and methods

Plant materials: We used 1 broad-leaved pioneer tree, *Alnus formosana*; 2 broad-leaved understory shrubs, *Ardisia crenata* and *Ardisia cornudentata*; and 4 ferns with different light-adaptation capabilities (ranked from high to low, *Pyrrosia lingus*, *Asplenium antiquum*, *Diplazium donianum*, *Archangiopteris somai*). Adult plants of *D. donianum* and *A. somai*, about 30 cm tall, were collected from Yuchi Township, Nantou County, Taiwan (23°49’N, 120°54’E, 560 m a.s.l.). One- to two-year-old *A. formosana* seedlings about 30–50 cm tall, and adult plants of the other 4 species, about 30 cm tall (*P. lingus*, *A. antiquum*) to 60 cm tall (*A. crenata*, *A. cornudentata*) were collected from Hueishun Forest Recreation Area, Ren’ai Township, Nantou County, Taiwan (24°05’N, 121°01’E, 800 m a.s.l.). All plants were collected in March 2010 and then transplanted to pots (16-cm diameter, 12-cm depth, 1 plant per pot for the 3 woody species and *A. antiquum*, and 1 rhizome with 3–4 leaves per pot for the other 3 ferns) filled with organic soil and maintained outdoors in the nursery at the Endemic Species Research Institute, Chichi Township, Nantou County, Taiwan (23°49’N, 120°48’E, 250 m a.s.l.). Materials were regularly watered and fertilized (half-strength Hoagland’s nutrient solution per month) and received up to 3 levels of light intensity, *i.e.*, 100%, 50% and 10% (beneath shade cloth) sunlight. Detailed information for each material and light condition are in Table 1. During the growth period of the materials (March 2010 to February 2011), the average hourly values of daily maximum photosynthetic photon flux density (PPFD) ranged from 1,296 to 1,456 μmol m^{-2} s^{-1} (Mar.−Aug.) and 1,150−750−1,171 μmol m^{-2} s^{-1} (Sept.−Dec.−Feb.) (data from the Endemic Species Research Institute).

Table 1. Tested species and their functional type, adapted light condition and light condition of cultivation (% of full sunlight) of materials. $^{\#}$ – measured under 15, 20, 25, and 30°C, the others were measured under 25°C.

Species	Functional type	Light condition of adaptation	Light condition of cultivation
Alnus formosana (Burk.) Makino	Broad-leaved pioneer tree	High light	100%$^{\#}$
Ardisia crenata Sims.	Broad-leaved understory shrub	Medium shade	50% and 10%
Ardisia cornudentata Mez.	Broad-leaved understory shrub	Medium shade	50% and 10%
Pyrrosia lingus (Thunb.) Farw.	Fern	Slight shade	100%$^{\#}$, 50% and 10%
Asplenium antiquum Makino.	Fern	Slight to medium shade	100%, 50% and 10%
Diplazium donianum (Mett.) Tard.-Blot.	Fern	Medium to heavy shade	50% and 10%
Archangiopteris somai Hayata.	Fern (endangered species)	Heavy shade	10%

Measurements were taken from September 2010 to February 2011, under eaves of a cottage at the Endemic Species Research Institute. At dawn of the measurement day, fully expanded younger leaves were selected and dark-adapted by covering with aluminum film until measurement. From 9:30 h to 15:00 h, photosynthesis, stomatal conductance and Chl fluorescence were measured by use of a portable, open-flow gas-exchange system (*LI-6400*, *LI-COR*, Lincoln, NE, USA) and an integrated fluorescence chamber head (*LI-6400-40*), stepwise from low to high levels of PPFD, *i.e.*, 0, 100, 200, 400, 800; 1,200; and 2,000 μmol m^{-2} s^{-1}. Leaf temperature was kept at 15, 20, 25, and 30°C for 100% sunlight-grown *A. formosana* and *P. lingus*, and 25°C for the other materials (Table 1). Plants were measured in the season with the climate temperature close to the leaf temperature. The 30°C measurement was in September, the 25°C measurement from October to November and the 15–20°C measurement from January to February. Throughout the measurements, CO_2 concentration and relative humidity in the chamber were kept at 350–400 μmol mol^{-1} (no control) and 75% (air entering chamber controlled by passing temperature-controlled water), respectively.

The values of CO_2 exchange, g_s, and Chl fluorescence were recorded every 2 min, until CO_2 exchange was stable (about 4 min in the dark, and 8−16 min under each level of illumination). P_G was calculated from P_N + dark respiration rate. The former is the stable CO_2-exchange rate under each level of PPFD, and the latter is the CO_2 exchange rate measured in the dark before illumination. The potential quantum efficiency of PSII (F_v/F_m) of dark-adapted leaves was calculated by $(F_m - F_0)/F_m$. F_0 and F_m, the minimal and maximal fluorescence, were determined by applying a weak pulse of light [0.1 μmol(quantum) m^{-2} s^{-1}] and a 0.8-s pulse of saturating flashes of approximately 6,000 μmol (quantum) m^{-2} s^{-1}, respectively. For leaves under each level of illumination, the actual PSII efficiency ($\Delta F/F_m$’) was calculated as (F_m’ – F)/F_m’. F and F_m’ are the actual and the maximal levels of fluorescence during illumination, respectively. The former was determined under each PPFD level of CO_2-exchange measurement, and the latter was determined by the same process as for F_m. ETR was calculated as $\Delta F/F_m$’ × PPFD × 0.5 × α (Maxwell and Johnson 2000). The average value of leaf absorption (α) for green leaves of 0.84 (Björkman and Demmig 1987) was used.

Statistics: Four to six leaves from four plants of each species grown each light condition were measured. Each leaf was taken as one replicate in statistical analyses. The light-response curve of photosynthetic rate was fitted by sigmoidal or hyperbolic equations. We used these equations to estimate the light-saturation point (PPFD of P_N to reach 90% of maximum, Hölscher *et al.* 2006, Huang *et al.* 2007). The other data were analyzed by linear or curve-linear regressions. All statistical analyses involved use of *Sigma Plot* version *10.0*.

Results

For the light-response curves of gas exchange measured at 25°C, the net photosynthetic CO_2-exchange rate (P_N) of all materials was increased in a curve-linear fashion with increasing PPFD (Fig. 1). The relation of P_N and PPFD for *A. somai* could be best fitted by the equation $y = a (1 - e^{-bx})$, and the others could be best fitted by the equation $y = ax/(b + x)$ ($y = P_N$, x = PPFD, $r^2 = 0.779 - 0.963$, $p<0.001$ for all). Woody plants or ferns adapted or acclimated to high light always had higher light-saturation point and maximal photosynthetic rate. *A. formosana*, a pioneer tree, had the highest P_N [23 μmol(CO_2) m^{-2} s^{-1} in maximum] and did not reach saturation under 2,000 μmol m^{-2} s^{-1} PPFD (Fig. 1*A*). *P. lingus*, a slight shade fern, had the second highest P_N and light-saturation point (Fig. 1*B*), followed by the other medium-shade plants, *A. antiquum* (Fig. 1*C*), *D. donianum* (Fig. 1*A*), *A. crenata* and *A. cornudentata* (Fig. 1*D*), whose maximal P_N and light-saturation point ranged from 3.1 to 6.7 μmol(CO_2) m^{-2} s^{-1} and 694 to 1,698 μmol m^{-2} s^{-1} PPFD , respectively. For *A. somai*, a heavy shade fern, the maximal P_N was only 2 μmol(CO_2) m^{-2} s^{-1}, and the curve reached saturation at about 300 μmol m^{-2} s^{-1} PPFD. For the effect of light condition on P_N of the same species, we found that all tested species always had higher photosynthetic rate and light-saturation point when grown in high sunlight (Fig. 1*A–D*). For PSII efficiency, before illumination, F_v/F_m of all dark-adapted leaves was about 0.8, with no significant difference among tested materials (Fig. 1). However, $\Delta F/F_m$' was decreased curve linearly with increasing PPFD and was greater for materials with low P_N. Thus, the light-response curve of ETR was near to the curve of P_N (data not shown), and ETR was closely related to P_N, even when data for low to high PPFD were merged (Fig. 3*B,D,F*). In addition, g_s also increased asymptotically with increasing PPFD. However, the increase in g_s was more rapid than that of P_N (Fig. 1*A–D* and *I–L*). Materials with higher P_N always showed higher g_s under high PPFD. However, *A. somai* and *D. donianum*, heavy- and medium- to heavy-shade adaptable ferns, had higher g_s than the other 2 tested ferns, and even lower P_N.

A. crenata and *A. cornudentata* had the highest ETR/P_G ratio (25.9–40.4) at 100 μmol m^{-2} s^{-1} PPFD (Fig. 2), and the ratio decreased greatly to 7.2–24.9 with PPFD increased to 400–800 μmol m^{-2} s^{-1}; then the ratio decreased gradually and was between 7.3–11.9 with PPFD increased to 2,000 μmol m^{-2} s^{-1} (Fig. 2*D*). For 50% sunlight-grown *A. antiquum*, the ratio of ETR/P_G under

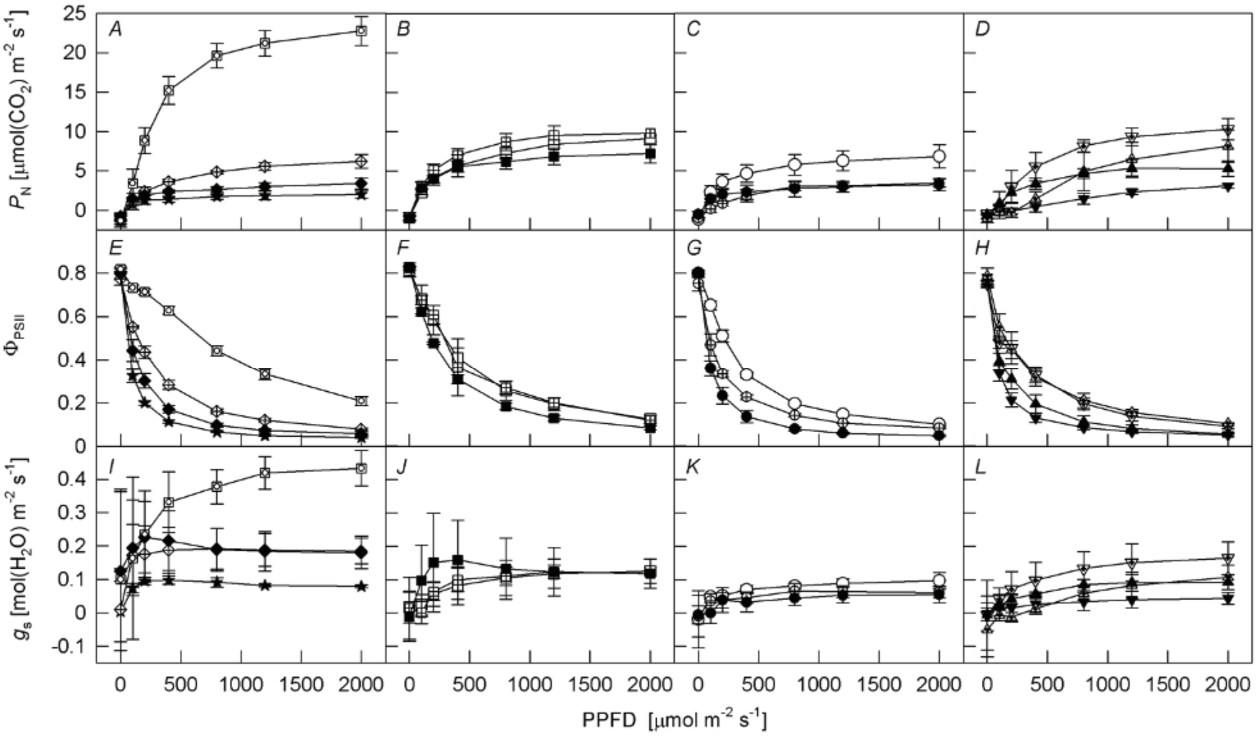

Fig. 1. The responses of net photosynthetic rate (P_N), PSII efficiency (Φ_{PSII}) and stomatal conductance (g_s) to photosynthetic photon flux density (PPFD) for *Alnus formosana* (▣), *Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), *Archangiopteris somai* (*stars*), *Ardisia cornudentata* (*upward triangles*) and *Ardisia crenata* (*downward triangles*) cultivated under 100% (*open symbols*), 50% (*cross symbols*) and 10% (*closed symbols*) of sunlight. Measurements were made from 0 to 2,000 μmol m^{-2} s^{-1} PPFD and 25°C. Each point represents the mean value of 4–6 leaves. Data are means ± SD.

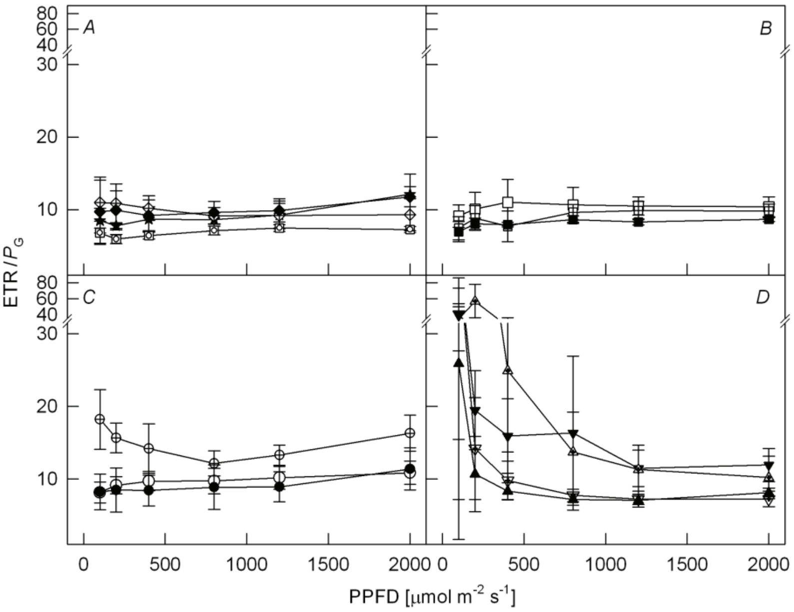

Fig. 2. The responses of ratio of electron transport rate to gross photosynthetic rate (ETR/P_G) to photosynthetic photon flux density (PPFD) for *Alnus formosana* (⊡), *Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), *Archangiopteris somai* (*stars*), *Ardisia cornudentata* (*upward triangles*) and *Ardisia crenata* (*downward triangles*) cultivated under 100% (*open symbols*), 50% (*cross symbols*) and 10% (*closed symbols*) of sunlight. Measurements were made from 100 to 2,000 μmol m^{-2} s^{-1} PPFD and 25°C. Each point represents the mean value of 4–6 leaves. Data are means ± SD.

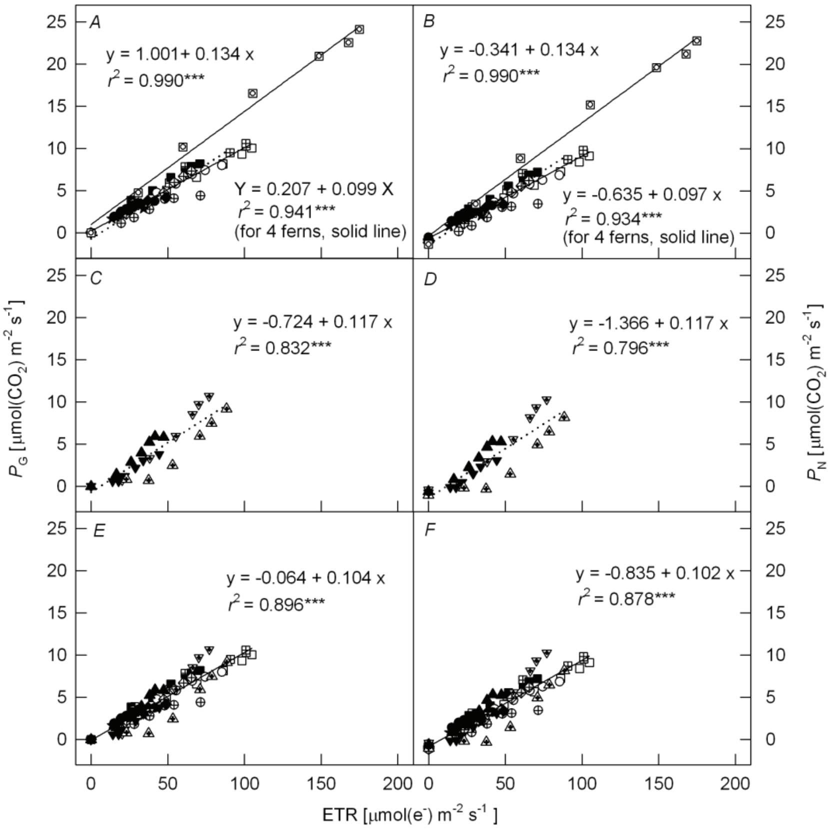

Fig. 3. The relationships between gross photosynthetic rate (P_G) and electron transport rate (ETR), and between net photosynthetic rate (P_N) and ETR for *Alnus formosana* (⊡), *Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), *Archangiopteris somai* (*stars*), *Ardisia cornudentata* (*upward triangles*) and *Ardisia crenata* (*downward triangles*) cultivated under 100% (*open symbols*), 50% (*cross symbols*) and 10% (*closed symbols*) of sunlight. Measurements were made under 25°C and from 0 to 2,000 μmol m^{-2} s^{-1} photon flux density. Each point represents the mean value of 4–6 leaves. The *dotted line* in panel *A* and *B* was copied from panels *C* and *D*, respectively. *** – p <0.001.

100 μmol m^{-2} s^{-1} PPFD was 18.2, but decreased slowly to 12.2 with PPFD increased to 800 μmol m^{-2} s^{-1} and then to 16.3 with 2,000 μmol m^{-2} s^{-1} PPFD (Fig. 2*C*). However, the other materials showed no significant difference in ETR/P_G ratio, even under different PPFD. Among them, *A. formosana* has the lowest ETR/P_G ratio (average 6.8 from 100 to 2,000 μmol m^{-2} s^{-1} PPFD). For fern species, except for the 50% sunlight-grown *A. antiquum*, the ETR/P_G ratio ranged from 7.4 (50% sunlight-grown *P. lingus*) to 12.1 (10% sunlight-grown *A. somai*) (Fig. 2*A–D*).

Because the ETR/P_G ratio was close for most tested materials, from low to high PPFD, ETR was strongly correlated with P_G (Fig. 3*A*,*C*) and even P_N (Fig. 3*B*,*D*). According to the ETR/P_G ratio, the P_G-ETR and P_N-ETR relations could be divided into 3 groups by species:

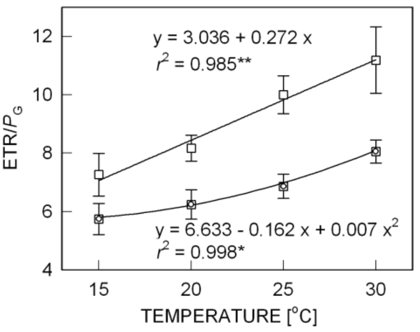

Fig. 4. The relationship between ratio of ETR/P_G and leaf temperature of 100% sunlight-grown *Alnus formosana* (⊡) and *Pyrrosia lingus* (□). Each point represents the mean value of 4–6 leaves measured at 100, 200, 400, 800; 1,200; and 2,000 µmol m^{-2} s^{-1} photon flux density. Data are means ± SD. * – $p < 0.05$, ** – $p < 0.01$.

A. formosana, 4 ferns and 2 understory shrubs. At the same levels of ETR, P_G and P_N was higher for *A. formosana* than did the 4 ferns and 2 understory shrubs. However, the regression lines between the 4 ferns and 2 understory shrubs were similar. Thus, even data obtained from the 2 groups (4 ferns and 2 understory shrubs) merged revealed stronger correlation between P_G and ETR (y = –0.064 + 0.104 x, r^2 = 0.896, $p<0.001$) (Fig. 3*E*) and between P_N and ETR (y = –0.835 + 0.102 x, r^2 = 0.878, $p<0.001$) (Fig. 3*F*).

At 15, 20, and 30°C, the ETR for the 2 tested species was still parallel to the variation in CO_2 fixation when measured under differential levels of PPFD (data not shown). However, the ETR/P_G ratio for both *P. lingus* and *A. formosana* was increased with increasing temperature from 15 to 30°C and could be fitted by first- and second-order equations, respectively (Fig. 4). Fig. 5 shows that the P_G estimated from these 2 regression equations [P_G = ETR/(3.036 + 0.272T) for *P. lingus*, and P_G = ETR/(6.633 – 0.162 T + 0.007 T^2) for *A. formosana*, where T is leaf temperature] was closely correlated with measured P_G (r^2 = 0.916 and 0.964, respectively, $p<0.001$). These relationships remained under 15–30°C and 0–2,000 µmol m^{-2} s^{-1} PPFD.

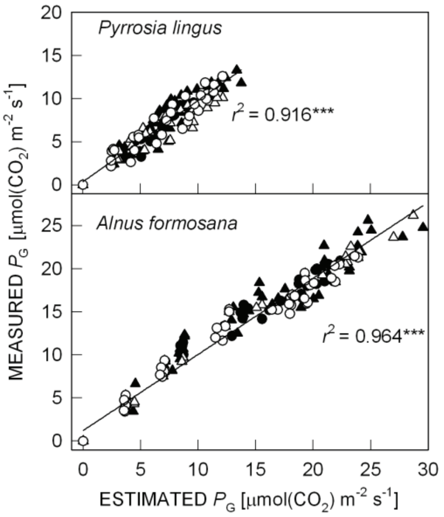

Fig. 5. Relationship between measured and estimated gross photosynthetic rate (P_G) [from P_G = ETR/(3.036 + 0.272 T) for *Pyrrosia lingus*, and P_G = ETR/(6.633 – 0.162 T + 0.007 T^2) for *Alnus formosana*, where T is leaf temperature]. ●, ▲, Δ and ○ were measured at 15, 20, 25, and 30°C, respectively; each point represents the value of each leaf measured under each level (0–2,000 µmol m^{-2} s^{-1}) of photon flux density. *** – $p < 0.001$.

Discussion

We tested 3 woody plants and 4 fern species with different light adaptation capabilities. The plant species adapted to- or acclimated to high light always had a higher light-saturation point and maximal photosynthetic rate (Fig. 1). Therefore, sun plants or sun leaves tend to efficiently use light energy by increasing their efficiency of CO_2 fixation, whereas shade plants or shaded leaves tended to have the opposite behavior. These results generally agree with previous results (*e.g.*, Givnish 1988, Pearcy and Sims 1994, Hölscher *et al.* 2006, Huang *et al.* 2007).

Some reports noted the light-response curve of g_s close to that of P_N (Yu *et al.* 2004, Huang *et al.* 2007), and late successional species or shade-grown plants showed low P_N and g_s (Hölscher *et al.* 2006, Huang *et al.* 2007). The physiological role of stomata is to prevent water loss and to facilitate CO_2 diffusion to mesophyll cells. Guard cells respond to intercellular CO_2 concentration, which is determined by atmospheric CO_2 and by the mesophyll assimilation rate (Vavasseur and Raghavendra 2005), which allows for a tight coupling between g_s and P_N. Our materials with high P_N under high PPFD always showed high g_s (Fig. 1), which agrees with the previous works. However, the P_N-g_s relationship may vary with species. Especially, ferns have low ability for detecting and responding to increases in CO_2 concentration, which leads to lower P_N/g_s ratio than those of angiosperms (Brodribb *et al.* 2005, Haworth *et al.* 2011). We found the same tendency for *A. somai* and *D. donianum*, heavy- and medium- to heavy-shade adaptable ferns (Fig.1). In addition, with PPFD increased from 0 to *ca.* 400 µmol m^{-2} s^{-1}, the increased rate of g_s was higher than that of P_N (Fig 1). Thus, in the well watered condition of the present study, g_s might be not a main limiting factor for photosynthesis, in combining all obtained data.

High light often leads to absorption of more photons than can be consumed (Stuhlfauth *et al.* 1990, Valladares and Pearcy 1997). To avoid the damage caused by excessive absorbed energy, plants often downregulate the efficiency of the photosystem, especially PSII (Demmig-Adams *et al.* 1996, Kato *et al.* 2003, Adams *et al.* 2004).

Thus, especially in C_4 plants, PSII efficiency (or ETR) is always parallel to variation in quantum yield of CO_2 fixation (or photosynthetic rate) under many cases (Earl and Tollenaar 1998, Kakani *et al.* 2008). In contrast, in C_3 plants, a significant correlation was found between ETR and P_G only under limited conditions because of the interference of photorespiration and other alternative pathways for electrons (Oberhuber *et al.* 1993, Cheng *et al.* 2001, Pérez-Torres *et al.* 2007, Ripley *et al.* 2007).

Although the rates of both CO_2 fixation and photorespiration may vary with temperature and PPFD, Ripley *et al.* (2007) indicated that C_3 *Alloteropsis semialata* could maintain a near-constant ratio of PSII efficiency to true quantum yield of CO_2 fixation under both photorespiratory and nonphotorespiratory conditions. In addition, this ratio measured under photorespiratory conditions was 1.8 times higher than that under nonphotorespiratory conditions. Therefore, under a constant temperature but varied PPFD conditions, the allocation of absorbed photons between CO_2 fixation and photorespiration may not be affected by photosynthetic rate. Thus, for some C_3 plants, a near-constant ratio of PSII efficiency to true quantum yield of CO_2 fixation or a significant linear correlation between quantum yield of CO_2 fixation (or photosynthetic rate) and PSII efficiency (or ETR) occurs under a constant temperature and nonphotorespiratory but varied PPFD (Pérez-Torres *et al.* 2007, Ripley *et al.* 2007, Sun *et al.* 2007). We found similar results in the present study. Moreover, with measurements under 25°C and 100–2,000 μmol m^{-2} s^{-1} PPFD, *A. formosana* and the 4 ferns, adapted to different light regimes, still showed a close (7–10) ETR/P_G ratio, even at differential levels of light intensity and maximal photosynthetic rate ranging from 2 to 23 μmol(CO_2) m^{-2} s^{-1} (Fig. 2). Thus, the photosynthetic rate of these 5 species may not lead to a different allocation between CO_2 fixation and alternative pathways of photons absorbed by the photosystem, even if P_G is influenced broadly by the PPFD of measurement or by species-specific morphological or physiological factors or light conditions of their growth. Of note, in previous (Pérez-Torres *et al.* 2007, Ripley *et al.* 2007, Sun *et al.* 2007) and the present studies, both CO_2 exchange and ETR were obtained simultaneously under a constant temperature but varied PPFD. Environmental and physiological factors did not differ during the measurement of the two characteristics. This situation may explain why we could obtain a close ETR/P_G ratio with low to high PPFD.

Even with measurements under the same conditions, the ratio of ETR/P_G or PSII efficiency/true quantum yield of CO_2 fixation may vary by species because of differences in allocation portion between CO_2 fixation and alternative electronic pathways (Oberhuber and Edwards 1993, Franco and Lüttge 2002, Cavender-Bares and Bazzaz 2004, Pérez-Torres *et al.* 2007, Sun *et al.* 2007). We found the ETR/P_G ratio lower for *A. formosana,* a sun woody plant, than for the other 6 tested species under each level of PPFD (Fig. 2). As well, *A. crenata* and *A. cornudentata*, both broad-leaved understory shrubs, showed a higher (20–57) ETR/P_G ratio with PPFD lower than 800 μmol m^{-2} s^{-1} (Fig. 2). Probably, Rubisco may not be fully activated during the early period of the measurements and resulted in the greater electron flow partitioning for the alternative electron pathways (Makino *et al.* 2002). This situation may explain the significantly higher ETR/P_G ratio during the early period of measurement. We found the ETR/P_G ratios similar for the 4 ferns throughout the light courses of measurements, and the ratio for *A. crenata* and *A. cornudentata* could be reduced close to that of the 4 ferns with PPFD higher than 800 μmol m^{-2} s^{-1}. Thus, even when merging data from the 2 groups (4 ferns and 2 understory shrub species), we found a stronger correlation between P_G and ETR (Fig. 3*E*). In addition, because of the small difference in dark respiration rate among species and light environment of their growth, we found the same tendency for the P_G-ETR relation as for the P_N-ETR relation (Fig. 3*F*). Thus, we may use these relations to estimate photosynthetic rate under different PPFD.

For C_3 plants, variations in temperature can affect the efficiency of both CO_2 fixation and alternative electron pathways (Hall and Keys 1983, Oberhuber and Edwards 1993, D'Ambrosio *et al.* 2006). However, with atmospheric CO_2 and O_2 concentrations, the temperature response of ETR/P_G ratio (or ETR/P_N and Φ_{PSII}/Φ_{CO_2}) varies by species. Oberhuber and Edwards (1993) reported that, in a broad temperature range (15–35°C), the Φ_{PSII}/Φ_{CO_2} ratio of *Flaveria pringlei* was increased with increasing temperature. In contrast, D'Ambrosio *et al.* (2006) pointed out that the ETR/P_N of *Beta vulgaris* was unaffected by temperature under 5–25°C but was increased with increasing temperature from 30 to 35°C. In addition, Kubien and Sage (2004) reported no variation in ETR/P_N ratio of 14/10°C (day/night)-grown *Calamogrostis canadensis* when measured at 5–35°C. Yet, this ratio of 26/22°C-grown *C. canadensis* increased with increasing temperature from 5 to 35°C. We found the ETR/P_G ratio for the 2 tested species increased with increasing temperature from 15 to 30°C but also found specific variation of the temperature response of the ETR/P_G ratio. Nevertheless, the temperature responses of the ETR/P_G ratio in *P. lingus* and *A. formosana* could be fitted by first- and second-order equations, respectively (Fig. 4). These equations contain only 2 easily obtained dynamic indicators, ETR and leaf temperature. Therefore, they could be used to estimate photosynthetic rate under different temperature and PPFD conditions. From the close correlation between the estimated and measured P_G, under different seasonal temperature (15–30°C) and 0–2,000 μmol m^{-2} s^{-1} PPFD conditions (Fig. 5), we conclude that these empirical regression models, developed from temperature responses of the ETR/P_G ratio could be used to simulate dynamic variation of leaf scale photosynthetic rate at different temperature and PPFD conditions. However, this empirical regression model might vary by species, because of different temperature responses of ETR/P_G ratio. In addition, this empirical regression model was unsuitable for some species, such as *A. crenata* and *A. cornudentata*, whose ETR/P_G ratio varied with PPFD.

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