Energy Quenching and Photoinhibition during Light Induction in Four Fern Species Adapted to Different Light Regimes

四種不同光適應性蕨類植物於光誘導期間之能量消散與光抑制

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Abstract

Four ferns with different light adaptation capabilities (ranking from high to low, *Pyrrosia lingus*, *Asplenium antiquum*, *Diplazium donianum*, *Archangiopteris somai*) were used to elucidate energy quenching and photoinhibition during light induction. Pot-grown materials received up to three levels of light intensity, *i.e.*, 100%, 50% and 10% sunlight, according to their light adaptation capabilities. At least six months after light acclimation, plant were illuminated with 50, 100, 300, 500, 1000 and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), respectively, for 30 min, and then dark-adapted for 30 min. Chlorophyll fluorescence were recorded just before and every 2–5 min during illumination and darkness. Results showed that the leaves measured under high-light intensity declined more in PSII efficiency and showed less reversion after dark adapted for 30 min (high photoinhibition). Even though four tested ferns had different capability of light adaptation, when compared to the same level of PPFD, the degree of photoinhibition was only divided into two groups. Among them, *P. lingus* utilized more sunlight with high photosynthetic capacity, while *A. antiquum* and *D. donianum* dissipated more excess light energy through energy-dependent quenching (qE). This showed that three species could maintain a low degree of photoinhibition, and this could possibly be attributed to their adaptation to direct sunlight and sunfleck. In contrast, *A. somai,*a heavy-shade adapted fern, showed low photosynthetic rate as well as qE, hence they had high level of excess energy and low ability of photoprotection, resulting in high degree of photoinhibition.

摘 要

本研究以 4 種不同光適應性之蕨類爲材料(光適應性由高至低分別爲石葦、山蘇花、細柄雙蓋蕨及台灣原始觀音座蓮)，探討在光度誘導下，其光保護及光抑制之機制。苗木分別培育於全光、50%遮光及 90%遮光 3 種不同光度環境下 6 個月後，以 50、100、300、500、1,000 及 2,000 μmol m^{-2} s^{-1} PPFD 光度各照射 30 分鐘，隨後再暗適應 30 分鐘，於光照前及光照和暗適應過程間每 2-5 分鐘皆紀錄一次葉綠素螢光值。結果顯示，越是陰性物種或於越高光照下測定時，其照光 30 分鐘後之光系統 II 效能($\Phi PSII_{L30}\%$)之下降程度越大，且在關燈 30 分鐘後之回復程度也越低，表示越容易受到光抑制。光抑制僅可大約分成二群。石葦因其光合作用能力較高可利用較多的光能，而山蘇花及細柄雙蓋蕨則可藉 qE 消散過多的光能，因此這三種受測之物種可維持低光抑制程度。可能這些物種爲了適應棲地直射光照或斑光而造成此等特性。相較之下臺灣原始觀音座蓮係重度偏好陰性之物種，其光合作用能力及 qE 皆較弱，光保護能力低下，因而導致光抑制的程度較高。

Key words: light adaptation, photosynthetic ability, photoprotection, energy quenching, photoinhibition, photosystem II·

關鍵詞：光適應、光合作用能力、光保護、能量消散、光抑制、光系統 II.

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Introduction

Sunlight is the energy source for plant photosynthesis and a major environmental factor influencing plant growth and distribution (Boardman 1977; Lambers *et al.* 1998). Plants may show reduced net carbon assimilation and growth if insufficiently exposed to light energy. However, under high irradiance, the light reaction may result in more photon absorbance than the carbon fixation can use, which often leads to photoinhibition of photosynthesis (Demmig-Adams *et al.* 1996; Kato *et al.* 2003; Morosinotto *et al.* 2003). This phenomenon damages the photosystem, especially PSII, because of the formation of toxic photoproducts such as O_2^-, H_2O_2, and OH^- (Demmig-Adams *et al.* 1996; Kato *et al.* 2003; Adams *et al.* 2004).

To protect the photosynthetic apparatus against photodamage, plants have developed several strategies to balance the captured and consumed photon energy. Xanthophylls cycle-dependent NPQ can play an important role in dissipating the excess energy as heat, thereby decreasing the efficiency of PSII (Demmig-Adams and Adams 1996; Li *et al.* 2000; Adams *et al.* 2004). Thus, the light energy absorbed by the photosystem can be quenched mainly by photochemical and non-photochemical processes, and the fraction of both types of quenching can be estimated by chlorophyll fluorescence variables (Demmig-Adams *et al.* 1996).

Under high light, with increasing light intensity, the proportion of excess light energy increases. The xanthophyll cycle and NPQ are often enhanced and PSII efficiency decreases. Nevertheless, PSII efficiency gradually recovers and NPQ decreases when light becomes weak (Demmig-Adams *et al.* 1996; Verhoeven et al. 1999). As well, when photosynthesis is inhibited by environmental or physiological factors, PSII efficiency may decrease because of increased excess light energy (Ghannoum *et al.* 2003; Adams *et al.* 2004; Weng 2009). At a lower layer of the canopy and understory, leaves commonly receive highly variable sunflecks. When leaves are exposed to a sudden increase in irradiance, the induction of photosynthesis requires several minutes to reach stability (e.g., Allen and Pearcy 2000; Wong *et al.* 2012b). This induction is always slower than the induction of PSII efficiency (Han *et al.* 1999; Bai *et al.* 2008; Wong *et al.* 2012b). At the initial stage of photosynthetic induction, the amount of absorbed photons is more than the CO_2 fixation can use because photosynthesis is not completely started; thus PSII efficiency decreases rapidly. Thereafter, PSII efficiency may increase and NPQ may decrease in parallel with the time-course increase in photosynthetic rate (Finazzi *et al.* 2004; Tausz *et al.* 2005; Bai *et al.* 2008; Wong *et al.* 2012b).

NPQ is a heterogeneous process. According to formation and dark relaxation kinetics, NPQ can be divided into at least three different components, namely, qE, qT and qI (Müller *et al.* 2001). qE is the fastest component, with induction and relaxation within seconds to minutes. It is related to xanthophyll cycle-dependent energy quenching. The second component, qT, which forms and relaxes in terms of minutes, is state-transition quenching. The third component, qI, develops when leaves are under prolonged exposure to highly excessive light and was originally ascribed to the photoinhibition of PSII. It needs more time to relax (Müller *et al.* 2001; Morosinotto *et al.* 2003; Finazzi *et al.* 2004; Kalituho *et al.* 2007; Zulfugarov *et al.* 2007).

Plant species adapted to different light regimes show differential photosynthetic characteristics. High-light-adapted species tend to have high photosynthetic rate and/or NPQ (Demmig-Adams *et al.* 2006; Golan *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; Demmig-Adams *et al.* 2006; Ballottari *et al.* 2007; Zhang *et al.* 2007; Dai *et al.,* 2009). However, in the same species, photosynthetic characteristics and NPQ may vary under different light conditions by the species' acclimation to different light regimes, and the allocation of absorbed light energy to photosynthesis versus NPQ differs. Demmig-Adams *et al.* (2006) suggested that the high-light-grown shade-intolerant species *Arabidopsis thaliana* and *Spinacia oleracea* could enhance their photosynthetic rate markedly to use more absorbed light energy. In contrast, shade-grown *Monstera deliciosa*, a shade-tolerant species, still showed low photosynthetic rate but enhanced xanthophyll cycle-dependent NPQ to disperse more absorbed light energy when transferred to high light. As well Ballottari *et al.* 2007, high-light-grown *Arabidopsis thaliana* showed high qE and low qI, which indicates the dissipation of more excess light energy and diminished photoinhibition, whereas low-light-grown *A. thaliana* showed the opposite results, with more photodamage (Ballottari *et al.* 2007).

Most ferns live in moist and shady environments. However, some species are able to grow with high irradiance. Light adaptation capabilities vary greatly among fern species (Saldaña *et al.* 2010; Wong *et al.* 2012a). Previously, we found that fern species adapted to different light regimes or the same species acclimated to different light intensity showed varied photosynthetic characteristics. Sun leaves of a slight-shade-adapted fern species showed higher photosynthetic rate and lower degree of photoinhibition than leaves of other medium- to heavy-shade-adapted fern species when exposed to high light (Wong *et al.* 2012b). However, the relation of energy quenching and photoinhibition in fern species adapted to different light regimes has not been studied in detail.

These fern plants distribute in different light environments show different photosynthetic rates in our previous researches. We use the parameter ETR as an indicator for estimating the photosynthetic rate of species across a wide taxonomic range and light adaptation and acclimation capability. By comparing the ETR and photosynthetic rate, *P. lingus,* which prefers habitats with higher light intensity, showed higher photosynthetic capability. *A. antiquum* and *D. donianum* showed lower photosynthetic capabilities, prefer distribution under shaded environments. *Arc. somai*, which prefers living under heavy shade, showed the lowest photosynthetic capability (Wong *et al.*, 2012a ; Wong *et al.*, 2014). Found by our previous results, the photosynthetic capabilities could be associated with light environment adaptations for these fern species. In this study, we used four fern species with different light adaptation capabilities in energy quenching and photoinhibition in leaves induced by different light intensity. For the purpose to examine that the fern species possessing higher photosynthetic rate or higher qE could diminish the excess light energy to prevent photoinhibition.

Materials and methods

Plant materials

We used four ferns with different light-adaptation capabilities (ranking from high to low: *Pyrrosia lingus*, *Asplenium antiquum*, *Diplazium donianum*, *Archangiopteris somai*). Adult plants, about 30 cm tall, were collected from central Taiwan (23°49′–24°05′N, 120°54′–121°01′E, 560–800 m a.s.l.), *Pyrrosia lingus*, which grows on the ground or trunks as an epiphyte, distributes in the gap or the border of the forest, palisades or other habitats with slight shade at the altitude range of about 0–2000 m a.s.l. *Asplenium antiquum*, which prefers habitats with slight to medium shade, distribute on the ground or the trunks under the forest canopy at an altitude range at about 0–1500 m a.s.l. *Diplazium donianum*, which prefers habitats with medium to heavy shade than *A. antiquum*, distributes on the soil or the rocks in the understory of the forests at an altitude range at about 0–1500 m a.s.l. *Archangiopteris somai*, which possesses very limited ecological amplitude than other fern species, prefers habitats with heavy shade and high relative humidity. In Taiwan, this species could be found in only two locations: Yuchi Township, Nantou County (23°56′N, 120°54′E, 700–800 m a.s.l., with mean annual temperature at about 21℃ and average annual rainfall about 2,200 mm) and Wulai Dist., New Taipei City (24°51′N, 121°32′E, 400–500 m a.s.l., with mean annual temperature at about 17℃ and average annual rainfall about 2,900 mm). The yellow soil in these habitats is deeply covered under dense canopy and contains abundant organic matters (Wong *et al.* 2012a ; Lin *et al.* 2015). Then the plants were transplanted to pots (16-cm diameter, 12-cm depth, 1 plant per pot for *A. antiquum* and 1 rhizome with 3–4 leaves per pot for the other three 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 one to three light intensities according to the light condition of their habitat: (1) complete sunshine, namely, 100% sunlight; (2) filtered 50% sunshine beneath a shade cloth, namely, 50% sunlight; and (3) filtered 90% sunshine beneath a shade cloth, namely, 10% sunlight.

The two slight- to medium-shade ferns, *P. lingus* and *A. antiquum*, received 10%, 50% and 100% sunlight. One medium-to-heavy shade fern, *D. donianum,* received both 10% and 50% sunlight; and one heavy-shade fern, *Arc. somai*, received 10% sunlight.

Measurements

At least six months after transplanting, the chlorophyll fluorescence of the fully expanded youngest leaves was measured. At nightfall the day before the measurement, potted materials were dark-adapted overnight (room temperature ~25 °C). On the measurement day, potted materials were placed in a growth chamber (*F-360DN*) at 25 °C. First, chlorophyll fluorescence of dark-adapted leaves was measured (see below) under darkness. Then leaves were exposed to luminescent lamps (halogen lamp and optical fiber) stepwise from low to high light, *i.e.*, 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} for 30 min. Chlorophyll fluorescence were recorded every 2 min during illumination. After illumination, leaves were dark-adapted for 30 min, and chlorophyll fluorescence was recorded every 2 min for 10 min, then every 5 min for 20 min.

Chlorophyll fluorescence was measured by using a portable chlorophyll fluorometer (*PAM-2100, Walz,* Germany). For dark-adapted leaves, F_o and F_m, the minimal and maximal fluorescence, respectively, were determined by applying a weak pulse of light (0.1 μmol photon m^{-2} s^{-1}) and a 0.8-s pulse of saturating flashes of approximately 6,000 μmol m^{-2} s^{-1}, respectively. For leaves under each level of illumination, F was determined for each PPFD level, and F_m’ was determined by the same process as for F_m. F and F_m’ are the actual and maximal levels, respectively, of fluorescence during illumination.

Calculation and statistical analysis

The potential quantum efficiency of PSII (F_v/F_m) was calculated as $(F_m - F_o)/F_m$; the actual PSII efficiency (ΦPSII) was calculated as $(F_m' - F)/F_m'$; and NPQ was calculated as $F_m/F_m' - 1$. qE and qI were calculated as $F_m/F_m' - F_m/F_{mD2}$ and $F_m/F_{mD30} - 1$, respectively. F_{mD2} and F_{mD30} is F_m with dark-adaptation for 2 and 30 min after illumination,. respectively (Bilger and Björkman 1990; Demming-Adams et al. 1996; Maxwell and Johnson 2000; Müller et al. 2001). Data are mean ± SE for 4 to 6 leaves from 4 plants for each species grown under each light condition. Each leaf was considered one replicate for statistical analyses. Statistical analyses involved use of Sigma Plot v10.0 (Systat Software, Point Richmond, CA, USA). $P<0.05$ was considered statistically significant.

Result

The F_v/F_m for all overnight dark-adapted leaves was ~0.8. When these leaves were suddenly exposed to 50 to 2,000 μmol m^{-2} s^{-1} PPFD, the ΦPSII decreased rapidly during the first 2 min of illumination, then increased with increasing illumination time almost to a stable level within ~5 to 20 min of illumination (Fig. 1). The steady ΦPSII value decreased with increasing light intensity. At 50 to 1,000 μmol m^{-2} s^{-1} PPFD, steady ΦPSII was in the approximate high to low order in *P. lingus*, *A. antiquum*, *D. donianum* and *Arc. somai* at the same PPFD. At 2,000 μmol m^{-2} s^{-1} PPFD, the ΦPSII for *P. lingus*, *A. antiquum* and *D. donianum* was ~0.2 to 0.3, whereas that for *Arc. somai* was < 0.1. In addition, low-light-grown plants always had lower steady ΦPSII than high-light-grown plants within the same species. When leaves were exposed to 50–500 μmol m^{-2} s^{-1} PPFD for 30 min, the relative values of ΦPSII (ΦPSII_{L30}%, F_v/F_m value for each treatment before illumination set to 100%) decreased near-linearly with increasing PPFD (Fig. 2A–D). *Arc. somai* showed the greatest decrease in ΦPSII_{L30}%, followed by *D. donianum* and *A. antiquum*, and *P. lingus* showed the least decrease. With increasing PPFD from 500 to 2,000 μmol m^{-2} s^{-1}, the decrease in ΦPSII_{L30}% gradually slowed to reach a steady state at > 1,000 μmol m^{-2} s^{-1} PPFD. At 2,000 μmol m^{-2} s^{-1} PPFD, *D. donianum*, *A. antiquum* and *P. lingus* remained at 30% to 40% of ΦPSII_{L30}% but *Arc. somai* at only about 10%.

After the light was turned off, with leaves exposed to 50 to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, F_v/F_m increased sharply within 2 min, then slowly reached a steady state within 2 to 15 min (Fig. 2). This steady F_v/F_m did not differ among plants grown under different light conditions as compared with the same species and at the same level of PPFD (Fig. 1). When illuminated leaves were dark-adapted for 30 min, the relative value of F_v/F_m [F_v/F_{mD30}%, F_v/F_m value for each treatment before illumination set to 100%] decrease with increasing PPFD (Fig. 2 E–H). Negative correlation relationships between F_v/F_{mD30}% and PPFD were observed for all tested species, and the descending slope of the regression equation was lower for *P. lingus*, *A. antiquum* and *D. donianum* than *A. somai* (0.003–0.004 vs. 0.006, Table 1).

Table. 1, Regression analyses for the association of relative values of photosystem II efficiency (F_v/F_{mD30}%, F_v/F_m value for each treatment before illumination set to 100%) and photoinhibitory quenching (qI) with photosynthetic photon flux density (PPFD) of four fern species under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} PPFD for 30 min.

Species	F_v/F_{mD30}%-PPFD			qI-PPFD		
	a	b (×10^2)	R^2	A	b (×10^3)	R^2
Pyrrosia lingus	97.648	–0.382	0.793***	0.145	0.116	0.792***
Diplazium donianum	96.253	–0.324	0.857***	0.114	0.116	0.848***
Asplenium antiquum	96.202	–0.395	0.915***	0.122	0.135	0.802***
Archangiopteris somai	90.601	–0.604	0.880**	0.172	0.309	0.928**

Data from Figs. 2 and 3 a and b: constant and regression coefficient of regression equation (Y = bX + a, Y is F_v/F_{mD30}% or qI, X is PPFD), respectively; ** and ***:$P < 0.01$ and $P < 0.001$, respectively.

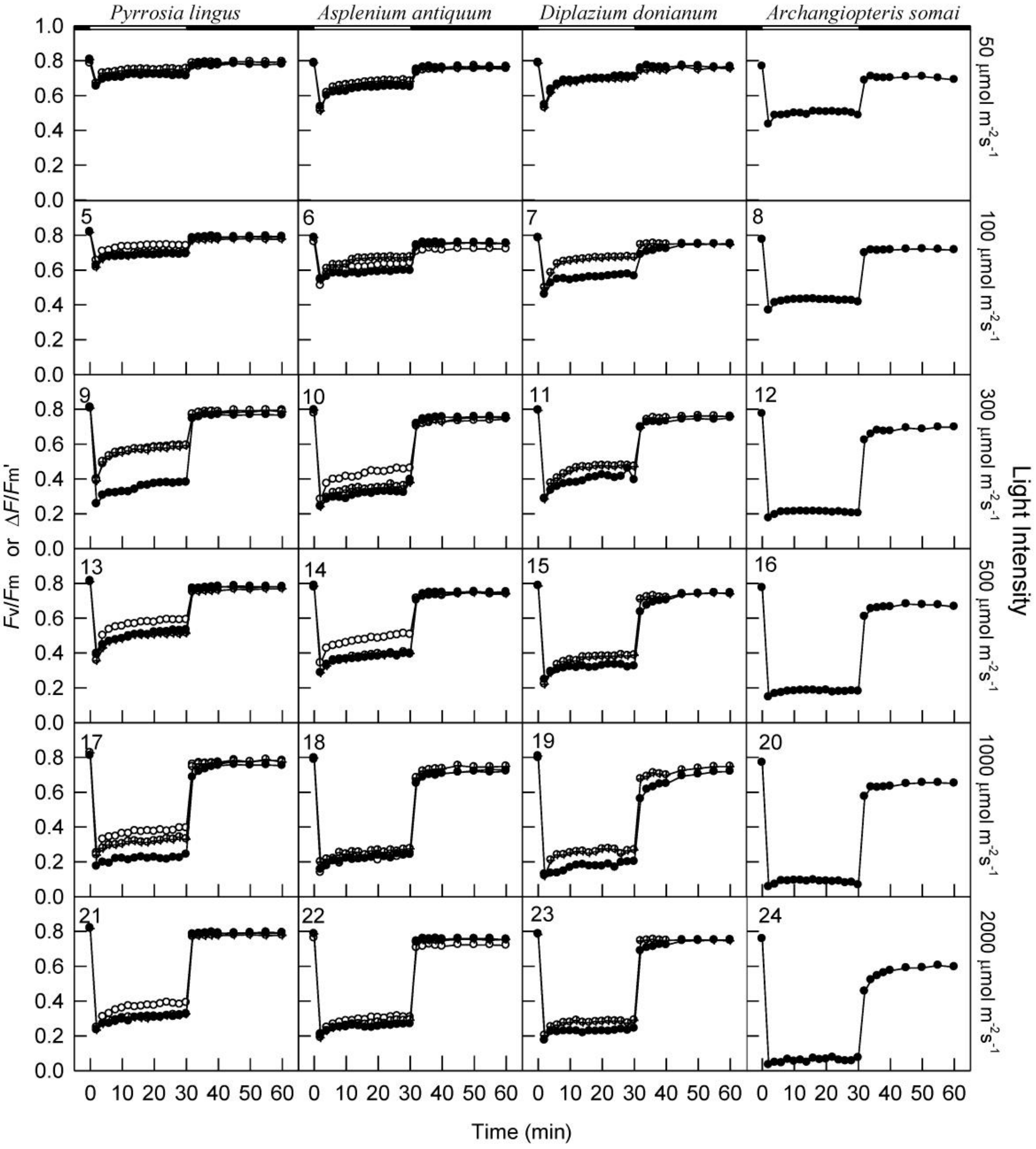

Fig. 1, Time course of illumination and darkness and variation in photosystem II efficiency [F_v/F_m under darkness and $\Delta F/F_m$' (ΦPSII) under illumination] for four fern species cultivated under 100% (○), 50% (⊕) and 10% (●) sunlight. Variables were measured under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density. Data are mean ± SE (n = 4). ■: darkness; □: illumination

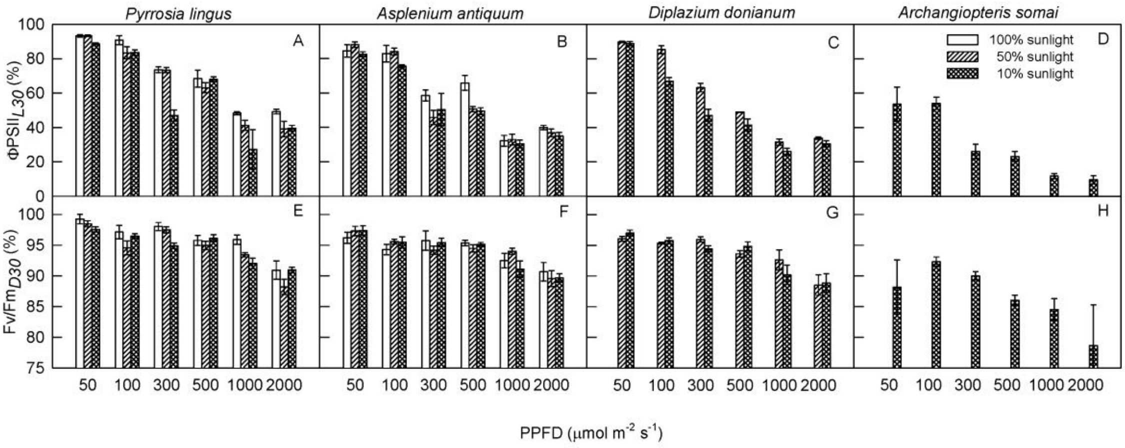

Fig. 2, The relative values of photosystem II efficiency in leaves of four fern species under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 30 min ($\Phi PSII_{L30}$(%)), then dark-adapted for 30 min (F_v/F_{mD30}%). Materials were cultivated under 100%, 50% and 10% sunlight for at least six months. Values are means ± SE; PSII efficiency of each treatment before illumination was set to 100%. [data of E–H are from Weng and Wong (2015)]

When leaves were suddenly exposed to 50–2,000 μmol m^{-2} s^{-1} PPFD for 30 min, NPQ of all treatments increased with increasing PPFD (Fig. 3A–D). The increasing pattern of qE with increasing PPFD was similar to NPQ but increased slower than NPQ for the heavy-shade-adapted fern *Arc. somai* (Fig. 3E–H). qI increased linearly with increasing PPFD for all species, and *Arc. somai* had a higher increasing slope than the other three species (0.0003 vs. 0.0001) (Fig. 3I–L and Table 1). NPQ was positively correlated with qE and qI when leaves were illuminated with 50 to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min. The slope for qE-NPQ regression was higher for *P. lingus*, *A. antiquum* and *D. donianum* than *Arc. somai* (0.724, 0.735 and 0.675 respectively, vs. 0.226) but lower for qI-NPQ regression (0.146, 0.109 and 0.104, respectively vs. 0.416; Fig. 4).

After leaves were illuminated with 50 to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, F_v/F_{mD30}% was negatively correlated with qI when combining data from each species grown under each light condition (R^2 = 0.784, P<0.001) (Fig. 5), so photoinhibition increased linearly with increasing qI for the four species negatively. As well, F_v/F_{mD30}% for *Arc. somai* located at the lower side of the regression line when qI was < 0.4. Thus, at low light intensity, *Arc. somai* showed greater photoinhibition than the other species even with the same qI.

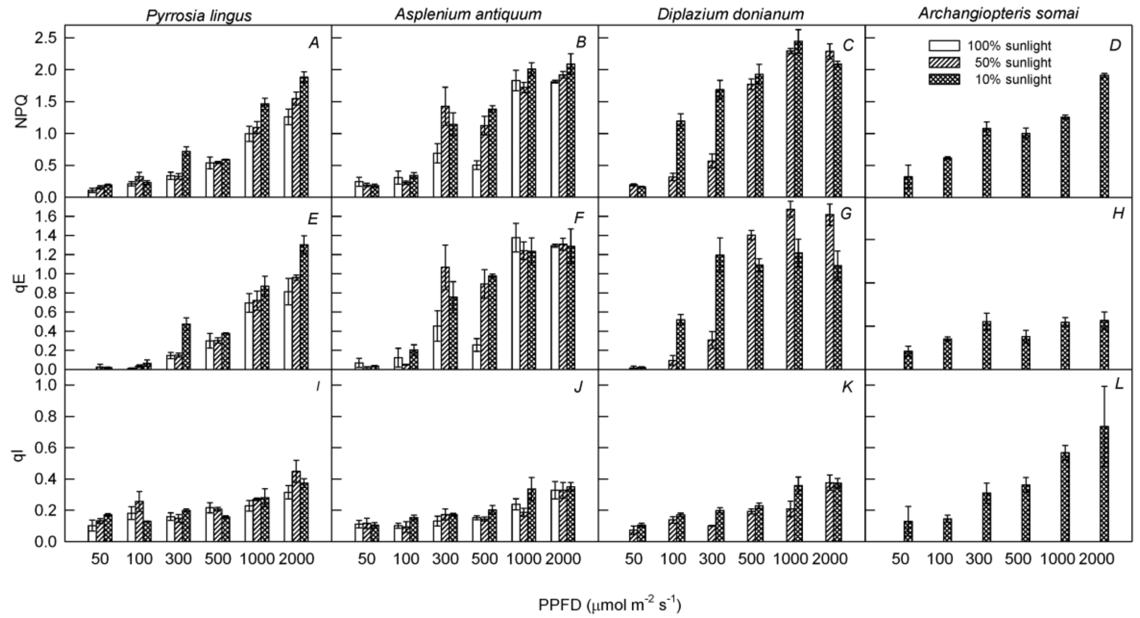

Fig. 3, Variation in non-photochemical quenching (NPQ), energy-dependent quenching (qE) and photoinhibitory quenching (qI) in leaves of four fern species under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 30 min. Materials were cultivated under 100%, 50% and 10% sunlight for at least six months. Values are means ± SE. [data of E–L are from Weng and Wong (2015)]

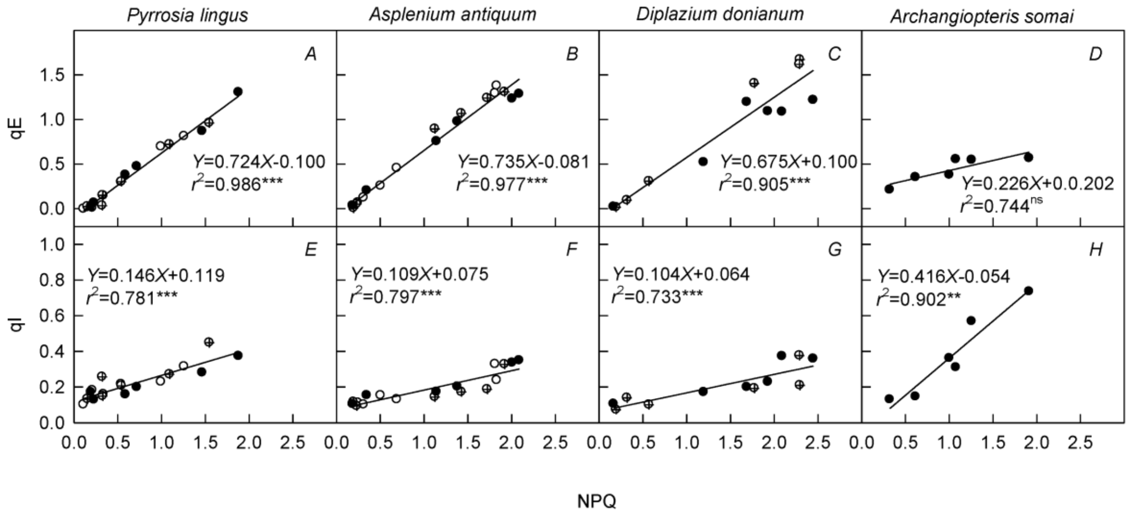

Fig. 4, Regression analysis of non-photochemical quenching (NPQ) and energy-dependent quenching (qE), and NPQ and photoinhibitory quenching (qI) in leaves of four fern species under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density for 30 min. Materials were cultivated under 100% (○), 50% (⊕) and 10% (●) sunlight for at least six months. **, *** and ns:$P < 0.01$, $P < 0.001$ and not significant.

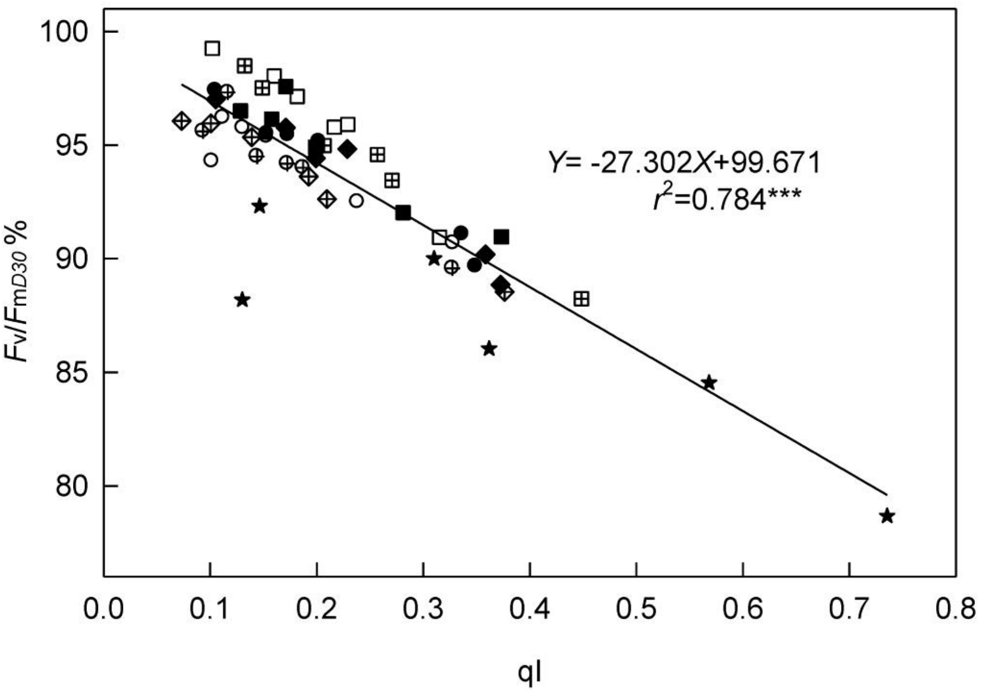

Fig. 5, The correlation of photoinhibitory quenching (qI) and degree of photoinhibition (F_v/F_{mD30}%, relative F_v/F_m value when leaves were illuminated for 30 min then dark adapted for 30 min, Fv/Fm value of each treatment before illumination set to 100%) in leaves of four fern species under 50, 100, 300, 500, 1,000 and 2,000 μmol m^{-2} s^{-1} PPFD for 30 min. Materials [*Pyrrosia lingus* (squares), *Asplenium antiquum* (circles), *Diplazium donianum* (diamonds) and *Archangiopteris somai* (stars)] were cultivated under 100% (open symbols), 50% (within the cross) and/or 10% (closed symbols) sunlight for at least six months. ***:$P < 0.001$.

Discussion

To understand the energy quenching and photoinhibition of fern species during light induction, we examined four ferns adapted to different light regimes. The F_v/F_m for all overnight-dark-adapted leaves was ~0.8 (Fig. 1), indicated that materials did not experience long-term photoinhibition before light induction (Björkman and Demmig 1987). When these dark-adapted leaves were suddenly exposed to 50 to 2,000 μmol m^{-2} s^{-1} PPFD, the ΦPSII decreased rapidly during the first 2 min of illumination, then increased with increasing time to a stable level. The transient decline in ΦPSII at the initial stage of light induction was mainly due to the downregulation of PSII efficiency (Finazzi *et al.* 2004; Kalituho *et al.* 2007; Zulfugarov *et al.* 2007). Because light induction of PSII efficiency is more rapid than that of CO_2

assimilation (Han *et al.* 1999; Bai et al. 2008; Wong *et al.* 2012b), the energy may be absorbed more at the early stage of photosynthetic induction. To avoid the damage caused by excess energy absorbed, PSII efficiency was sharply downregulated to a low level, with greater downregulation under high light because of greater excess absorbed energy (Thiele *et al.* 1998; Tausz *et al.* 2005; Bai *et al.* 2008).

The excess absorbed energy may decrease with increasing photosynthetic rate over time (Mott *et al.* 1997; Finazzi *et al.* 2004), which led to an increase in ΦPSII with increasing illumination time to a stable level (Fig. 1). The steady value of ΦPSII decreased with increasing light intensity and explains why the excess absorption of light energy often leads to decreased the PSII function (Demmig-Adams *et al.* 1996; Kato *et al.* 2003; Morosinotto et al. 2003; Tu *et al.* 2012). For the difference in steady value of ΦPSII among fern species under the same level of PPFD, at 50 to 500 μmol m^{-2} s^{-1} PPFD, the low-light-adapted species showed lower $\Phi PSII_{L30}$% than those adapted to high light (Fig. 2A–D). This finding might be due to the former species having lower photosynthetic rate than the latter species (Wong *et al.* 2012a, b) and thus decreased requirement for light energy (Murchie and Horton 1998; Yamashita *et al.* 2000; Cai *et al.* 2005; Rodriguez-Calcerrada *et al.* 2007). To avoid the damage caused by excess energy absorbed, the former species may downregulate their PSII efficiency more than the latter species.

Under high light, leaves may absorb more photons than photosynthesis can use, and this excessively absorbed energy often leads to photoinhibition (Demmig-Adams *et al.* 1996; Kato *et al.* 2002; Morosinotto *et al.* 2003). Species adapted to or leaves acclimated to high light tend to show low photoinhibition at the final state of light induction (Wong *et al.* 2014). However, even though our four fern species had different light adaptation capability, the descending slope of F_v/F_{mD30}% with increasing light intensity led to the classification of only two groups when overnight dark-adapted leaves were suddenly exposed to 50 to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min (Fig. 2E–H). For *Arc. somai*, a heavy-shade-adapted fern, the descending slope was higher than for the other three test ferns that were slight-shade-adapted (*P. lingus*), slight- to medium-shade-adapted (*A. antiquum*) and medium- to heavy-shade-adapted (*D. donianum*). Thus, with light intensity > 1,000 μmol m^{-2} s^{-1} PPFD, *Arc. somai* had the lowest ΦPSII and F_v/F_{mD30}%, so the fern was more vulnerable to photoinhibition than the other three species.

These differences among species might be due to the species-specific variation in photosynthetic rate as well as qE and qI, which are components of NPQ. qE is related to the xanthophylls cycle-dependent energy quenching and represents the major portion of photoprotection, whereas qI is related to photoinhibition and represents the major portion of damage of PSII (Müller et al. 2001; Morosinotto *et al.* 2003; Kalituho *et al.* 2007). Our study also showed that qI was negatively correlated to F_v/F_{mD30}% (Fig. 5).

Therefore, leaves with high qI showed severe photoinhibition.

Plants have several strategies to balance the capture and use of photon energy to avoid photoinhibition. Demmig-Adams *et al.* (2006) noted that high-light-adapted annual species used more sunlight, with high photosynthetic capacity. Conversely, a medium- to low-light-adapted perennial species, *Monstera deliciosa*, had low photosynthetic capacity but showed high xanthophylls cycle-dependent NPQ to avoid photoinhibition in full sunlight. Our previous results (Wong *et al.* 2012a) indicated that the photosynthetic rate of tested species was in the order of *P. lingus*, slight-shade-adapted (maximal P_N (net photosynthetic rate) was about 9 μmol(CO_2) m^{-2} s^{-1},), > *A. antiquum* (maximal P_N was about 6 μmol(CO_2) m^{-2} s^{-1}), slight- to medium-shade-adapted, > *D. donianum* (maximal P_N was about 5 μmol(CO_2) m^{-2} s^{-1}), medium- to heavy-shade-adapted > *Arc. Somai* (maximal P_N was only about 2 μmol(CO_2) m^{-2} s^{-1}), heavy-shade-adapted. In contrast, the order for the xanthophyll cycle-dependent qE was *D. donianum* > *A. antiquum* > *P. lingus* > *Arc. somai* when leaves were exposed 300 to 1,000 μmol m^{-2} s^{-1} PPFD for 30 min (Fig. 3E–H). Therefore, *P. lingus* could use more sunlight with high photosynthetic capacity and did not need high qE to dissipate the excess light energy. In contrast, even *A. antiquum* and *D. donianum* showed lower photosynthesis ability than *P. lingus* (Wong *et al.* 2012a, b), but they could dissipate more excess light energy via qE (Fig. 3F, G). Thus, these two species could maintain a similar level of qI as *P. lingus* under the same level of PPFD (Fig. 3I–K).

When leaves were exposed to a sudden increase in irradiance, the two shade-adapted ferns, *A. antiquum* and *D. donianum*, showed diminished qI through qE, probably because of their adaptation to sunflecks. In contrast, *Arc. somai* showed low photosynthetic rate (Wong *et al.*, 2012a, b) and qE (Fig. 3H and 4D) but high qI (Fig. 3L, 4H and 5). The species might retain more excess light energy, which leads to more drastic photoinhibition (Fig. 2H and 5). In addition, *Arc. somai* showed a lower slope for the qE-NPQ regression but higher slope for the qI-NPQ regression than the other three tested species (Fig. 4). For *Arc. somai*, the major portion of NPQ and the decrease in PSII efficiency during light induction might be caused by qI but less due to downregulation of PSII efficiency through photoprotection mechanisms. This characteristic is probably related to its adaptation to heavy shade.

We found that when overnight dark-adapted leaves were suddenly exposed to 50 to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, the species-specific differences in photoinhibition were closely related to the species' energy utilization and quenching with adaptation to different light regimes. *P. lingus*, a slight-shade-adapted fern, could use more sunlight with high photosynthetic capacity, whereas *A. antiquum*, a slight- to medium-shade-adapted fern, and *D. donianum*, a medium- to heavy-shade-adapted fern, could dissipate more excess light energy through qE.

Thus, under the same level of PPFD, these three species could maintain a low degree of qI. In contrast, *Arc. somai*, a heavy-shade-adapted fern, showed low photosynthetic rate as well as qE and so a high level of excess energy and low photoprotection ability, for a high degree of qI. In addition, photoprotection of PSII is affected by antioxidation (Golan *et al.*, 2006; Murchie and Niyogi 2011). However, this feature needs further investigation.

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