DOI: 10.1007/s11099-014-0023-z PHOTOSYNTHETICA 52 (2): 272-280, 2014

Light induction of nonphotochemical quenching, CO_2 fixation, and photoinhibition in woody and fern species adapted to different light regimes

S.-L. WONG[*], M.-Y. HUANG[**], C.-W. CHEN[*] and J.-H. WENG[***,+]

Division of Botany, Endemic Species Research Institute, Chichi, Nantou, Taiwan[*]
Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan[**]
Graduate Institute of Ecology and Evolutionary Biology, China Medical University, Taichung, Taiwan[***]

Abstract

We aimed to find out relations among nonphotochemical quenching (NPQ), gross photosynthetic rate (P_G), and photoinhibition during photosynthetic light induction in three woody species (one pioneer tree and two understory shrubs) and four ferns adapted to different light regimes. Pot-grown plants received 100% and/or 10% sunlight according to their light-adaptation capabilities. After at least four months of light acclimation, CO_2 exchange and chlorophyll fluorescence were measured simultaneously in the laboratory. We found that during light induction the formation and relaxation of the transient NPQ was closely related to light intensity, light-adaption capability of species, and P_G. NPQ with all treatments increased rapidly within the first 1–2 min of the light induction. Thereafter, only species with high P_G and electron transport rate (ETR), *i.e.*, one pioneer tree and one mild shade–adapted fern, showed NPQ relaxing rapidly to a low steady-state level within 6–8 min under PPFD of 100 μmol(photon) m^{-2} s^{-1} and ambient CO_2 concentration. Leaves with low P_G and ETR, regardless of species characteristics or inhibition by low CO_2 concentration, showed slow or none NPQ relaxation up to 20 min after the start of low light induction. In contrast, NPQ increased slowly to a steady state (one pioneer tree) or it did not reach the steady state (the others) from 2 to 30 min under PPFD of 2,000 μmol m^{-2} s^{-1}. Under high excess of light energy, species adapted to or plants acclimated to high light exhibited high NPQ at the initial 1 or 2 min, and showed low photoinhibition after 30 min of light induction. The value of fastest-developing NPQ can be quickly and easily obtained and might be useful for physiological studies.

Additional key words: light adaptation; NPQ; photoinhibition; photosynthetic induction; photosynthetic rate

Introduction

Sunlight is the energy source for plant photosynthesis and one of the major environmental factors for growth and distribution of plant species (Boardman 1977, Lambers *et al*. 1998). Under high irradiance, more photons may be absorbed than the carbon reaction can use; such excess of absorbed energy often leads to reduced efficiency of photosystems, especially PS II (Demmig-Adams *et al*. 1996, Kato *et al*. 2003, Adams *et al*. 2004). Plants use photoprotective mechanisms to counteract the harmful effects of excessive photon absorption. In the photoprotection, NPQ plays an important role: it quenches excess energy and dissipates it safely as heat (Müller *et al*. 2001, Murchie and Niyogi 2011).

NPQ is a heterogeneous process. According to formation and dark relaxation kinetics, it can be divided into at least 3 different components, namely, q_E, q_T, and q_I (Müller *et al*. 2001). The most slowly forming component, q_I, shows very long relaxation time (in a range of hours)

Received 20 May 2013, *accepted* 7 August 2013.
[+]Corresponding author; fax: +886 4 22071507, e-mail: jhweng@mail.cmu.edu.tw
Abbreviations: ETR – electron transport rate; F_v/F_m – potential quantum efficiency of PSII; $F_v/F_m\%^{HL}$ – relative value of potential quantum efficiency of PSII in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min; HL – high light (2,000 μmol m^{-2} s^{-1} PPFD); LL – low light (100 μmol m^{-2} s^{-1} PPFD); NPQ – nonphotochemical quenching; NPQ_{1min}^{LL} and NPQ_{1min}^{CF} – NPQ in leaves exposed to 100 μmol m^{-2} s^{-1} PPFD under ambient CO_2 and 0 inlet CO_2 concentration for 1 min, respectively; NPQ_{1min}^{HL} and NPQ_{2min}^{HL} – NPQ in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration for 1 min and 2 min, respectively; NPQ_{D30min}^{HL} – NPQ in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, then darkness for 30 min; P_G – gross photosynthetic rate; PPFD – photosynthetic photon flux density; q_E − xanthophyll cycle-dependent energy quenching; q_I − photoinhibitory quenching; q_T − state-transition quenching.

under darkness. It develops when leaves are under prolonged exposure to highly excessive light and it was originally ascribed to the photoinhibition of PSII. The second component, q_T, is state-transition quenching, which forms and relaxes within tens of minutes. The component q_E is the fastest forming and reversible part of NPQ; it relaxes within seconds to minutes (Müller *et al*. 2001).

Recently, another type of q_E quenching was proposed. This quenching is induced within the first 1–2 min after dark-adapted plants are exposed to nonsaturating light, then it decreases rapidly during the light induction (Finazzi *et al*. 2004, Kalituho *et al*. 2007, Zulfugarov *et al*. 2007). This transient q_E depends on PsbS protein and low lumen pH (Finazzi *et al*. 2004, Kalituho *et al*. 2007, Zulfugarov *et al*. 2007). The transient q_E is also largely modulated by zeaxanthin (Kalituho *et al*. 2007, Nilkens *et al*. 2010) and by PSII antenna size (Zulfugarov *et al*. 2007). In contrast, the latter q_E formation (within 10–15 min) is correlated with the synthesis and epoxidation of zeaxanthin (Nilkens *et al*. 2010).

The induction and relaxation of NPQ strongly depends on light intensity and on various physiological aspects. Under the low light intensity, such as PPFD of 30–100 μmol m^{-2} s^{-1}, NPQ generates only transiently, peaking after about 60 to 100 s and then relaxing to a low steady-state level. Under such light intensities, the maximum NPQ increases with increasing PPFD, PsbS protein, and zeaxanthin content, as well as lumen acidification (Finazzi *et al*. 2004, Kalituho *et al*. 2007, Zulfugarov *et al*. 2007). Under the medium light intensity (such as 150–500 μmol m^{-2} s^{-1} PPFD), NPQ increases rapidly within about 2 min, then increases gradually to the stable steady state. Leaves exposed to photoinhibitory irradiance show rapidly increasing NPQ, within about 2 min, which then increases gradually until the end of light induction (Kalitoho *et al*. 2007, Zulfugarov *et al*. 2007). In addition, under high light intensity (such as PPFD higher than 1,500 μmol m^{-2} s^{-1}), plants show enhanced NPQ and/or photosynthetic rate to dissipate excessive light energy (Bailey *et al*. 2004, Demmig-Adams *et al*. 2006). Thus, the formation and relaxation of NPQ may be influenced by the rate of photosynthesis. When dark-adapted leaves are exposed to a sudden increase in irradiance, photosynthetic CO_2 fixation is delayed before the maximal rate of assimilation is achieved, thus, NPQ may be high at the beginning of the light induction. Thereafter, relaxation of the transient NPQ is parallel to the time-course increase of photosynthetic activity during low light induction (Finazzi *et al*. 2004).

However, photosynthetic characteristics and NPQ of plants may vary by species adapted to different light regimes and the allocation of absorbed light energy to photosynthesis *vs.* NPQ differs. Sun plants or leaves tend to exhibit high photosynthetic rate and/or NPQ (Scholes *et al*. 1997, Demmig-Adams *et al*. 2006, Golan *et al*. 2006). The sun species use full sunlight for photosynthesis to a much greater extent than do species adapted to a broad light range. Conversely, the latter species show a greater NPQ capacity in full sun than the sun species (Scholes *et a1*. 1997, Demmig-Adams *et al*. 2006). Thus, even in leaves under the same PPFD, the relationship between photosynthetic rate and NPQ may vary by a species and a level of PPFD.

Ecophysiological studies require knowledge of photosynthetic characteristics of different species and under different environments. However, the induction and relaxation of NPQ under constant light intensity has been studied mainly in wild-type *Arabidopsis* and in its mutants (Ballottari *et al*. 2007, Kalituho *et al*. 2007, Zulfugarov *et al*. 2007, Nilkens *et al*. 2010). It was rarely examined in other species and it was not compared to photosynthetic rates (Finazzi *et al*. 2004). Moreover, the fastest-developing NPQ component is an important mechanism to protect the photosystem at high light intensity (Müller *et al*. 2001, Murchie and Niyogi 2011). In physiological studies, this type of NPQ might be fast and easy to measure. However, the relationships among NPQ, photoinhibition, photosynthetic rate, and light-adaptation capacity in broad taxonomic species have not been studied in details.

In this study, we used 3 woody species and 4 ferns adapted to different light regimes to compare: (*1*) the light induction of NPQ and CO_2 fixation at high and low light intensities; and (*2*) the relationship between the fastest-developing NPQ component and light-adaption capability as well as photoinhibition of species.

Materials and methods

Plant material: The plants were the same as in Wong *et al*. (2012). *i.e*., 1 broad-leaved, pioneer tree, *Alnus formosana* (Burkill) Makino; 2 broad-leaved, understory shrubs, *Ardisia crenata* Sims and *Ardisia cornudentata* Mez; and four ferns with different light-adaptation capabilities, ranked from high to low: *Pyrrosia lingus* (Thunb.) Farw., *Asplenium antiquum* Makino, *Diplazium donianum* (Mett.) Tard. -Blot., and *Archangiopteris somai* Hayata. In March 2010, adult plants of four ferns (about 30 cm tall) and two understory shrubs (about 60 cm tall), as well as 1–2--year-old seedling, about 30–50 cm tall of *A. formosana* were collected from central Taiwan. All plants were transplanted to pots (16-cm diameter, 12-cm depth, one plant per pot for the three woody species and *A. antiquum*, and one rhizome with 3–4 leaves per pot for the other three ferns) filled with organic soil and maintained outdoors in the nursery of the Endemic Species Research Institute, Chichi Township, Nantou County, Taiwan (23°49′N, 120°48′E, 250 m a.s.l.). Plants were regularly watered and fertilized (half-strength Hoagland's nutrient solution per month) and received up to two levels of light intensity by two levels of sunlight [*i.e.*, 100% and 10%

(beneath shade cloth)] according to the light condition of their habitat (*see* the text table below). During the growth period, the average hourly values of daily maximum PPFD ranged from 1,296−1,456 μmol m^{-2} s^{-1} (Mar−Aug) and 1,150−770 μmol m^{-2} s^{-1} (Sep−Nov) (data from the Endemic Species Research Institute).

Species	Type of plant	Illumination [% of sunlight]
A. formosana	broad-leaved pioneer tree	100
A. crenata	broad-leaved understory shrub	10
A. cornudentata	broad-leaved understory shrub	10
D. donianum	medium- to heavy-shade fern	10
A. somai	heavy-shade fern	10
P. lingus	mild-shade fern	100 and 10
A. antiquum	mild- to medium-shade fern	100 and 10

Low light (LL) induction: Measurements were carried out in November 2011 in a laboratory at the Endemic Species Research Institute. At nightfall of one day before the measurement, potted plants were dark-adapted overnight (room temperature maintained at ~25°C). On the next day, fully expanded, younger leaves were selected for measurements. First, CO_2 exchange and Chl fluorescence of the dark-adapted leaves were measured simultaneously by a portable, open-flow gas-exchange system (*LI-6400*, *LI-COR*, Lincoln, NE, USA) with an integrated fluorescence chamber head (*LI-6400-40*) under darkness. Then, the leaves were exposed to 100 μmol m^{-2} s^{-1} of PPFD by the light source of the fluorescent chamber head for 20 min, and the values of CO_2 exchange and Chl fluorescence were recorded every 1 min during initial 4 min, then every 2 min. Throughout the measurements, leaf temperature and relative humidity in the chamber were kept at 25°C and 75% (air entering chamber controlled by passing temperature-controlled water), respectively, under two different CO_2 concentrations: 350–400 μmol mol^{-1} (no control) and 0 μmol mol^{-1} inlet CO_2 (air entering chamber was passing through temperature-controlled saturated KOH solution).

In the dark-adapted leaves, the minimal and maximal fluorescence, F_0 and F_m, 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. In leaves under illumination, F and F_m' were the actual and maximal levels of fluorescence, respectively. The former was determined under induction light (100 μmol m^{-2} s^{-1} PPFD), and the latter was determined similarly as F_m.

High light (HL) induction: Measurements were done from August to October 2010 and in November 2011 in a laboratory at the Endemic Species Research Institute using the same equipment as above. In the 2010, overnight dark-adapted leaves were exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, then dark-adapted for 30 min. The values of CO_2 exchange and Chl fluorescence were recorded just before and every 2 min during illumination. Chl fluorescence under darkness was recorded at 2, 4, and 30 min after the end of light induction. In November 2011, the overnight dark-adapted leaves were exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 4 min. The values of CO_2 exchange and Chl fluorescence were recorded just before and every 1 min during illumination. In both two periods, leaf temperature, CO_2 concentration, and relative humidity in the chamber were kept at 25°C, 350–400 μmol mol^{-1} (no control), and 75%, respectively, throughout the measurements. The Chl fluorescence parameters were obtained as during LL induction.

Calculation and statistical analysis: Gross photosynthetic rate (P_G) was calculated as the sum of net photosynthetic rate and dark respiration rate. The former was the CO_2 exchange rate under light, and the latter was the CO_2 exchange rate measured in the dark just before illumination. The potential quantum efficiency of PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$. NPQ and electron transport rate (ETR) were calculated as: $F_m/F_m' - 1$ and $\Delta F/F_m' \times PPFD \times 0.5 \times \alpha$, respectively (Maxwell and Johnson 2000). We used the mean value of 0.84 for leaf absorption (α) in green leaves (Björkman and Demmig 1987).

Data presented were obtained for each leaf (Figs. 3,4) or they presented the mean (the other figures) of 3–5 (0 inlet CO_2) and 4–6 (ambient CO_2) leaves from four plants of each species grown under both light condition. Each leaf was used as 1 replicate in statistical analyses. Data were analyzed by linear regression analysis using *Sigma Plot 10.0* (*Systat Software*, Point Richmond, CA, USA).

Results

The time-course variations of NPQ, P_G, and ETR under LL were shown in Fig. 1. Induction curves of P_G and ETR under HL were published previously (Wong *et al*. 2012). Thus, we showed the typical time-course variation in NPQ in Fig. 2*A* (obtained in 2010) and Fig. 2*B* (obtained in 2011 To compare the effect of photosynthetic rate on NPQ, we also showed typical data for P_G obtained at 2,000 μmol m^{-2} s^{-1} PPFD in 2010 in Fig. 2*C*.

When overnight dark-adapted leaves were suddenly exposed to light, NPQ, P_G, and ETR increased rapidly during the first 1–2 min of illumination at LL or HL. Thereafter, the relation among those three parameters and induction time varied greatly by species, light intensity, and CO_2 concentration. At LL and ambient CO_2 concentration, P_G and ETR increased with prolonging illumination time and became stable within 2–8 min. In species

with high P_G and ETR, NPQ was only transiently generated, peaking after 1 min of illumination, then relaxing rapidly to a low steady-state level within 6–8 min. In contrast, species with low P_G and ETR showed unrelaxed NPQ until the end of light induction (20 min), even though P_G and ETR reached a steady state within 1–6 min. When P_G was inhibited by low CO_2 concentration, NPQ was enhanced in all plants and increased rapidly during the first 1–2 min of illumination, with only mild or no relaxation on subsequent light induction. Under saturated light intensity (HL), NPQ increased rapidly within first 1–2 min of light induction, then increased slowly; it did not relax

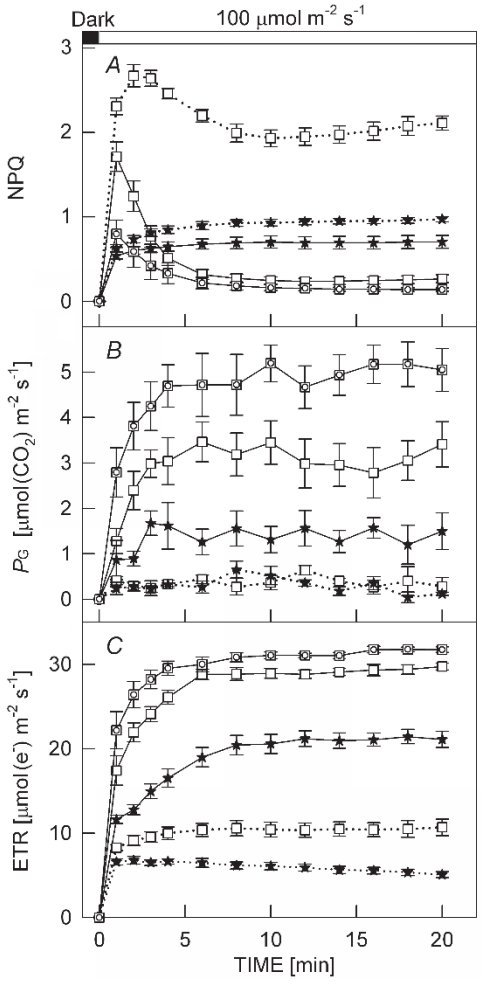

Fig. 1. Time course variation in nonphotochemical quenching (NPQ), gross photosynthetic rate (P_G), and electron transport rate (ETR) in *Alnus formosana* (⊡), *Pyrrosia lingus* (*squares*), and *Archangiopteris somai* (*stars*) cultivated under 100% (open symbols) and/or 10% (*closed symbols*) sunlight. Variables were measured at 25°C and 100 μmol m^{-2} s^{-1} photosynthetic photon flux density, at ambient (*solid lines*) and 0 inlet CO_2 (*dotted lines*) concentrations. Data are means ± SE [n = 3–5 (0 inlet CO_2) and 4–6 (ambient CO_2)].

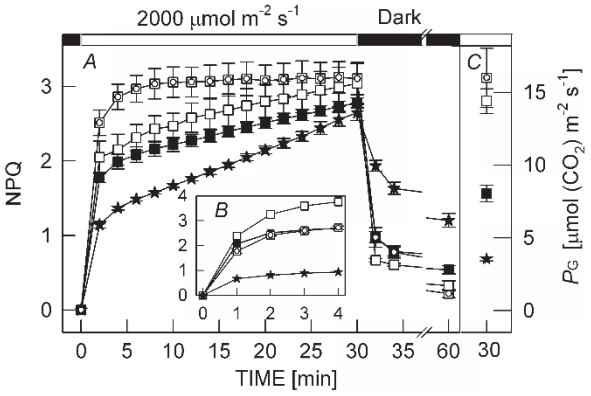

Fig. 2. Time course of illumination and darkness; and variation in nonphotochemical quenching (NPQ, *A*,*B*), and gross photosynthetic rate (P_G) at 30 min after the start of light induction (*C*) in *Alnus formosana* (⊡), *Pyrrosia lingus* (*squares*), and *Archangiopteris somai* (*stars*) cultivated under 100% (*open symbols*) and/or 10% (*closed symbols*) sunlight. Variables were measured under 25°C; 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux density and ambient CO_2 concentration. Data are means ± SE (n = 4–6). Panels *A* and *C* represent 2010 measurements [data of *C* are from Wong *et al*. (2012)]; panel *B* represents 2011 measurements.

until the end of light induction (30 min) in all tested species. Leaves with high P_G showed always high NPQ at the initial part of light induction, then it increased more slowly than in leaves with low P_G (Fig. 2).

The above results suggested that NPQ was closely associated with P_G and ETR. These relations existed not only among species but also among individual leaves within the same species. To elucidate this matter in more details, we used variables for single leaves to analyze the relations NPQ *vs.* P_G and NPQ *vs*. ETR at the initial (first 1 and 2 min under LL and HL induction, respectively) and final (20 and 30 min after the start of LL and HL induction, respectively) states of light induction. Under LL, leaves with low P_G and ETR, regardless of species characteristics or inhibition by low CO_2, showed always high NPQ, at both 1 min or 20 min after the start of light induction (Fig. 3). However, at given P_G and ETR, leaves of the pioneer tree, *A*. *formosana*, and 1 mild-shade fern (*P*. *lingus*) exhibited always the highest NPQ, followed by two understory shrubs (*A*. *crenata* and *A*. *cornudentata*), two medium- to heavy shade-adapted ferns (*A*. *antiquum* and *D*. *donianum*), and one heavy-shade-adapted fern (*A*. *somai*). Yet, at the final stage of light induction, the leaves with high P_G and ETR (P_{G20mim} and ETR_{20mim}) always showed high NPQ relaxation ($\Delta NPQ_{1–20min}$, NPQ value obtained at 1 min minus that obtained at 20 min after the start of light induction; Fig. 3*E*,*F*).

After 2 min of HL, P_G and ETR showed a weak, but significant, positive correlation with NPQ when data from all tested leaves were merged for regression analysis

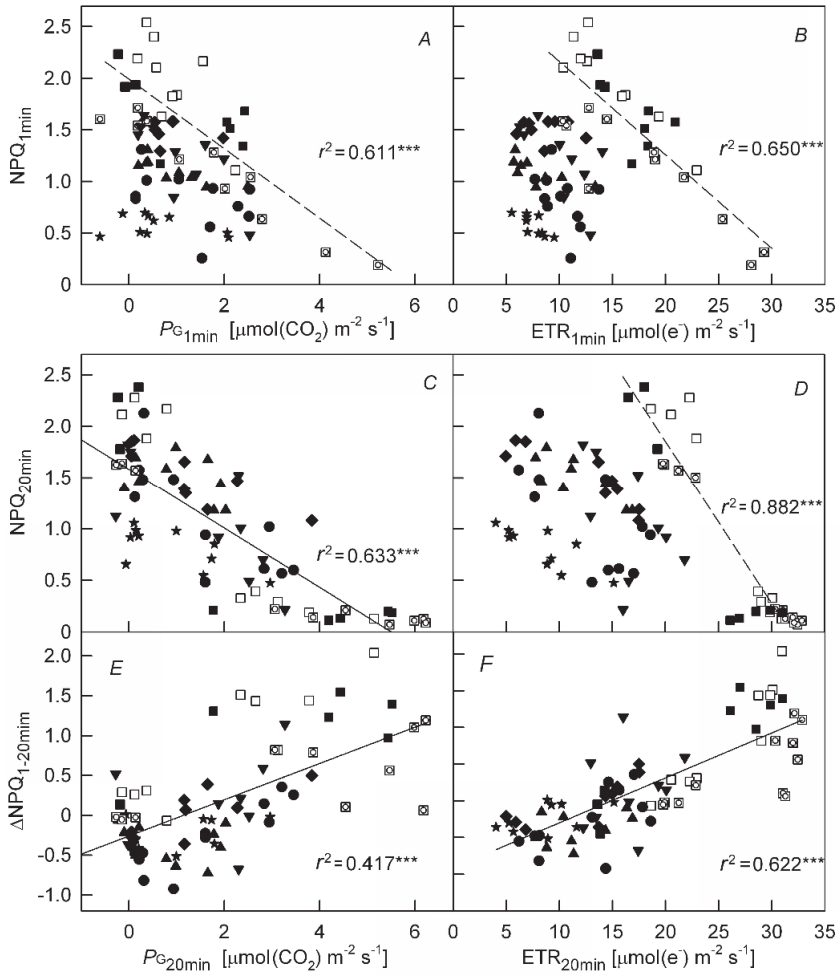

Fig. 3. Relation between nonphotochemical quenching (NPQ) and gross photosynthetic rate (P_G), and between NPQ and electron transport rate (ETR) in 3 woody species [*Alnus formosana* (⊡), *Ardisia crenata* (*downward triangles*), and *Ardisia cornudentata* (*upward triangles*)] and 4 fern species [*Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), and *Archangiopteris somai* (*stars*)] cultivated under 100% (*open symbols*) and/or 10% (*closed symbols*) sunlight. Variables were measured at 25°C, 100 μmol m^{-2} s^{-1} PPFD, and ambient or 0 inlet CO_2 concentrations. Each point represents the value for 1 leaf. *A*,*B*,*C*,*D*: data were obtained at 1 and 20 min, respectively, after the start of light induction. *E*,*F*: P_G and ETR were obtained 20 min after the start of light induction, and NPQ value is the difference in values obtained at 1 and 20 min after the start of light induction. *Solid* and *dotted* regression *lines* are data from all materials and from *A. formosana* (a pioneer tree) and *P. lingus* (a mild shade–adapted fern) combined for regression analysis, respectively. *** – p<0.001.

(Fig. 4*A*,*B*). In contrast, after 30 min of HL, NPQ was negatively correlated with P_G and ETR. However, the sun leaves of the pioneer tree and two ferns (*P. lingus*, adapted to mild-shade, and *A. antiquum*, adapted to mild- to medium-shade) had always higher NPQ at the given P_G and ETR (Fig. 4*C*,*D*). In most tested plants, the NPQs obtained at the initial stage of light induction (first 1 or 2 min) but under different light intensities or CO_2 concentrations significantly correlated with each other (Fig. 5). However, under LL and ambient CO_2 concentration, the NPQ for *A. formosana* was low because of high P_G (Fig. 3*A*). Thus, NPQ was located at the far left of the regression lines, when combining all tested plants for regression analysis (Fig. 5).

When the light was turned off, the NPQ relaxed sharply within 2 min, then decreased slowly in the leaves exposed to HL for 30 min (Fig. 2*A*). Sun leaves of one pioneer tree and two mild- to medium-shade–adapted ferns retained only 10% of NPQ, while the shade leaves retained from 45% (*A. somai*, a heavy- shade–adapted fern) to about 20% (two understory shrubs and one medium- to heavy shade–adapted fern) until they were in darkness for 30 min. The remaining NPQ (NPQ_{D30min}^{HL}) correlated negatively with that obtained at 2 min after the start of HL (NPQ_{2min}^{HL}) (Fig. 6). In addition, under HL or 0 inlet CO_2 concentration), species adapted to or leaves acclimated to HL tended to exhibit high NPQ at the initial stage (first 1 or 2 min) of light induction. They had high relative potential quantum efficiency of PSII ($F_v/F_m\%^{HL}$) when exposed to HL for 30 min and then darkened for 30 min (F_v/F_m of overnight dark-adapted leaves was 100%). Thus, photoinhibition tended to be low in the leaves with high NPQ obtained at the initial state of light induction (Fig. 7*B–D*). However, the NPQ of *A. formosana* under LL and ambient CO_2 concentration was located at the far left of the NPQ *vs.* $F_v/F_m\%^{HL}$ regression line, which indicated low NPQ (Fig. 7*A*).

Discussion

We used combined measurements to examine the light induction of P_G, ETR, and NPQ in several species adapted to different light regimes. The NPQ of all plants increased rapidly during the first 1–2 min of light induction

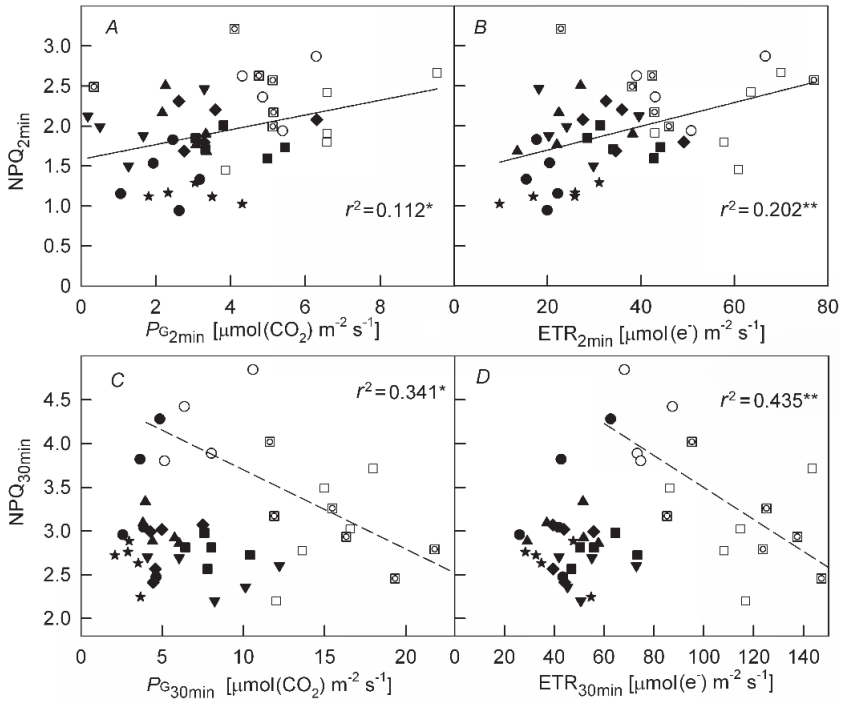

Fig. 4. Relation between nonphotochemical quenching (NPQ) and gross photosynthetic rate (P_G), and between NPQ and electron transport rate (ETR) in 3 woody species [*Alnus formosana* (▣), *Ardisia crenata* (*downward triangles*), and *Ardisia cornudentata* (*upward triangles*)] and 4 fern species [*Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), and *Archangiopteris somai* (*stars*)] cultivated under 100% (*open symbols*) and/or 10% (*closed symbols*) sunlight. Variables were measured at 25°C; 2,000 μmol m^{-2} s^{-1} photosynthetic photon flux, and ambient CO_2 concentration. Each point represents the value for 1 leaf. *A*,*B*,*C*,*D*: data were obtained at 2 and 30 min, respectively, after the start of light induction. *Solid* and *dotted* regression *lines* are data from all plants and from sun leaves of *A. formosana* (a pioneer tree), *P. lingus* (a mild-shade–adapted fern), and *A. antiquum* (a mild- to medium shade–adapted fern) combined for regression analysis, respectively. * – p<0.05, ** – p<0.01.

regardless of LL or HL and CO_2 conditions. However, thereafter, the relation between NPQ and induction time varied largely by species, as well as light intensity and CO_2 concentration during light induction (Figs. 1*A*, 2*A*).

Typically, transient q_E was found only under LL. Here, NPQ may peak at about 60 to 100 s after the start of illumination, then relaxes rapidly during the first 3–4 min of illumination, then slowly to a low steady-state level (Finazzi *et al*. 2004, Kalituho *et al*. 2007). In this study, only one pioneer tree (*A. formosana*) and one mild shade–adapted fern (*P. lingus*) showed marked transient NPQ under LL and ambient CO_2 concentration (Figs. 1*A*, 3*E*,*F*). As well, the formation and relaxation of the transient NPQ during LL induction were closely related to photosynthetic rate and ETR, despite variations in these characteristics were due to the differences of species, as well as different leaves in the same species, and CO_2 concentrations during measurement (Fig. 3). The leaves with high P_G and ETR tended to have low NPQ at both the initial (1 min after) and final (20 min after) stages of light induction and showed high NPQ relaxation from 1 to 20 min after the start of illumination. The leaves with low P_G and ETR, regardless of species or inhibition by low CO_2, showed slight or no NPQ relaxation with subsequent light induction. Therefore, NPQ relaxation under LL was closely associated with the utilization of light energy absorbed by the photosystems. The leaves with low P_G might require less light energy. To avoid the damage caused by excess of absorbed energy, leaves might disperse more energy through NPQ and thus they show slight or absent NPQ relaxation during light induction. By contrast, leaves with high photosynthetic rate have less superfluous light energy and they show lesser need to disperse energy through NPQ (Scholes *et al*. 1997, Demmig-Adams *et al*. 2006, Golan *et al*. 2006). Therefore, the NPQ decreases in parallel with the time-course increase in photosynthetic rate (Finazzi *et al*. 2004).

However, compared to the same levels of P_G and ETR, the rank of NPQ at both 1 and 20 min after the start of 100 μmol m^{-2} s^{-1} PPFD induction was as following pioneer tree and mild shade–adapted fern > broad-leaved understory shrubs and medium- to heavy shade–adapted ferns > heavy shade–adapted fern (Fig. 3*A*–*D*). Plants adapted or acclimated to high light tend to have high photosynthetic rate and/or NPQ (Scholes *et al*. 1997, Demmig-Adams *et al*. 2006, Golan *et al*. 2006). At the early state of LL induction, the maximum NPQ value increases with increasing PsbS protein and zeaxanthin content, as well lumen acidification (Finazzi *et al*. 2004; Kalituho *et al*. 2007, Zulfugarov *et al*. 2007). During the later stage of light induction (10–15 min), the zeaxanthin dependent q_E is NPQ component involved (Nilkens *et al*. 2010, Murchie and Niyogi 2011). Sun leaves have higher contents of PsbS and xanthophylls cycle pigments and form the higher ratio of zeaxanthin and antheraxanthin than shade leaves (Demmig-Adams *et al*. 2006, Golan *et al*. 2006, Ballottari *et al*. 2007, Yamazaki *et al*. 2007). Probably for this reason, the species more adapted to HL regimes showed higher NPQ at given P_G and ETR and thus they could dissipate excessive light energy more than species adapted to LL regimes.

At ambient CO_2 condition, P_G closely correlated with ETR (Figs. 1,2 and Wong *et al*. 2012). However, under 0 inlet CO_2, most leaves retained 50% to 70% of ETR, although P_G was inhibited to almost zero, as compared with the same material measured under the same PPFD but

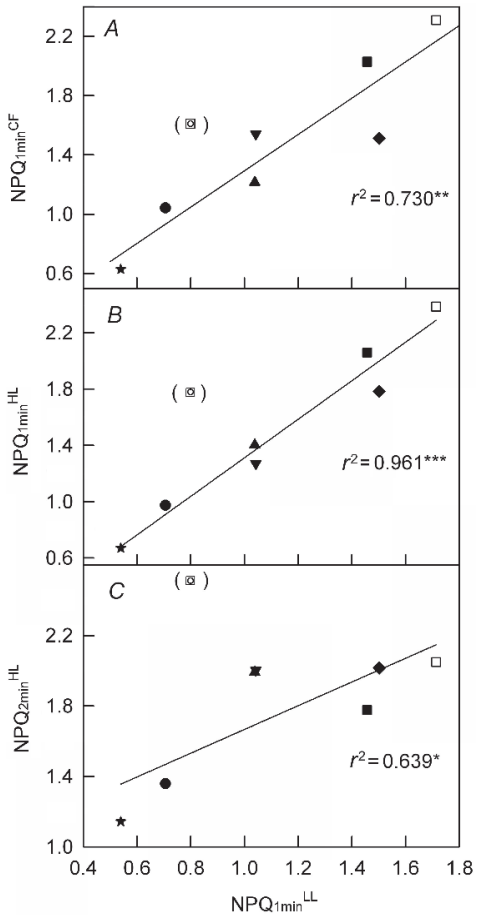

Fig. 5. Relation between nonphotochemical quenching (NPQ) in leaves exposed to 100 μmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration for 1 min (NPQ_{1min}^{LL}) and NPQ under other conditions (NPQ_{1min}^{CF} for leaves exposed to 100 μmol m^{-2} s^{-1} PPFD and 0 inlet CO_2 concentration for 1 min; NPQ_{1min}^{HL} and NPQ_{2min}^{HL} for leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration for 1 min and 2 min, respectively) in 3 woody species [*Alnus formosana* (▣), *Ardisia crenata* (*downward triangles*), and *Ardisia cornudentata* (*upward triangles*)] and 4 fern species [*Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), and *Archangiopteris somai* (*stars*)] cultivated under 100% (*open symbols*) and 10% (*closed symbols*) sunlight. Each point represents the mean of 3–5 (0 inlet CO_2) and 4–6 (ambient CO_2) leaves. *, ** and *** – p<0.05, 0.01 and 0.001, respectively, excluding data for *Alnus formosana* ▣.

ambient CO_2 condition (Fig. 1*B*,*C* and data not shown). The electrons might flow to alternative energy sinks, other than CO_2 fixation, such as photorespiration (Peterson 1994), water–water cycle (Asada 1999), cyclic electron flow within PSII (Miyake and Okamura 2003), and nitrogen assimilation (Robinson 1990).

At HL and ambient CO_2 concentration, NPQ increased rapidly within the first 1–2 min of light induction, but transient q_E could not be found in all tested plants (Fig. 2*A* and data not shown). This result was similar to previous findings (Ballottari *et al*. 2007, Thaipratum *et al*. 2009,

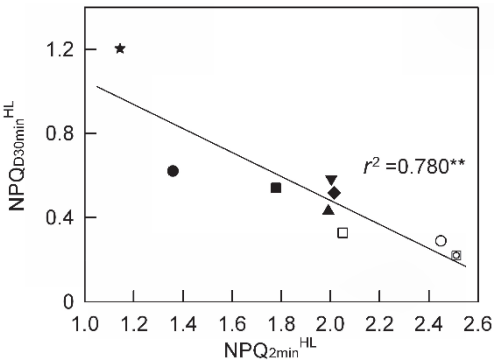

Fig. 6. Relation between nonphotochemical quenching (NPQ) in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 2 min (NPQ_{2min}^{HL}) and NPQ in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min, then darkness for 30 min (NPQ_{D30min}^{HL}) in 3 woody species [*Alnus formosana* (▣), *Ardisia crenata* (*downward triangles*), and *Ardisia cornudentata* (*upward triangles*)] and 4 fern species [*Pyrrosia lingus* (*squares*), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), and *Archangiopteris somai* (*stars*)] cultivated under 100% (*open symbols*) and 10% (*closed symbols*) sunlight. Each point represents the mean of 4–6 leaves. ** – p<0.01.

Nilkens *et al*. 2010). Nevertheless, similarly to LL, species more adapted to HL regimes showed higher NPQ under the initial stage of HL induction. Thus, NPQ during the first 1 or 2 min of HL induction positively correlated with NPQ during the first 1 min of light induction under LL in most tested plants, when combining all tested species for regression analysis (Fig. 5*B*,*C*). However, the NPQ value was higher under HL than under LL as compared with the same species at the initial state of light induction. The NPQ formation at the initial state might be promoted by light intensity (Kalituho *et al*. 2007); as well as by high contents of PsbS protein and zeaxanthin, when leaves were induced at both low (Finazzi *et al*. 2004, Kalituho *et al*. 2007, Zulfugarov *et al*. 2007) and high light intensities (Ballottari *et al*. 2007, Thaipratum *et al*. 2009). As well, similar values of NPQ under HL could be found under LL but 0 inlet CO_2 (Fig. 5). Both conditions might manifest excessive light energy, either due to HL or inhibited photosynthesis.

The NPQ of the pioneer tree, *A. formosana*, measured during the first 1 min under LL and ambient CO_2 concentrations, was located at far left of the regression lines (Fig. 5). Therefore, *A. formosana* showed a relatively low NPQ value under such conditions. The reason could be that *A. formosana* had the higher P_G and thus lower NPQ than the other tested species (Fig. 3*A*). In contrast, under LL and 0 inlet CO_2, as well as HL and ambient CO_2, *A. formosana* could exhibit a high NPQ at the initial stage of the light induction, because photosynthesis was inhibited by the low CO_2 concentration (Fig. 3*A*) or photosynthesis was not yet activated enough (Fig. 4*A*). Sun leaves have high photosynthetic rate and can dissipate the absorbed excess light energy through NPQ (Scholes *et al*. 1997, Demmig-Adams *et al*. 2006, Golan *et al*. 2006), but their photosynthesis is not yet activated enough at the initial

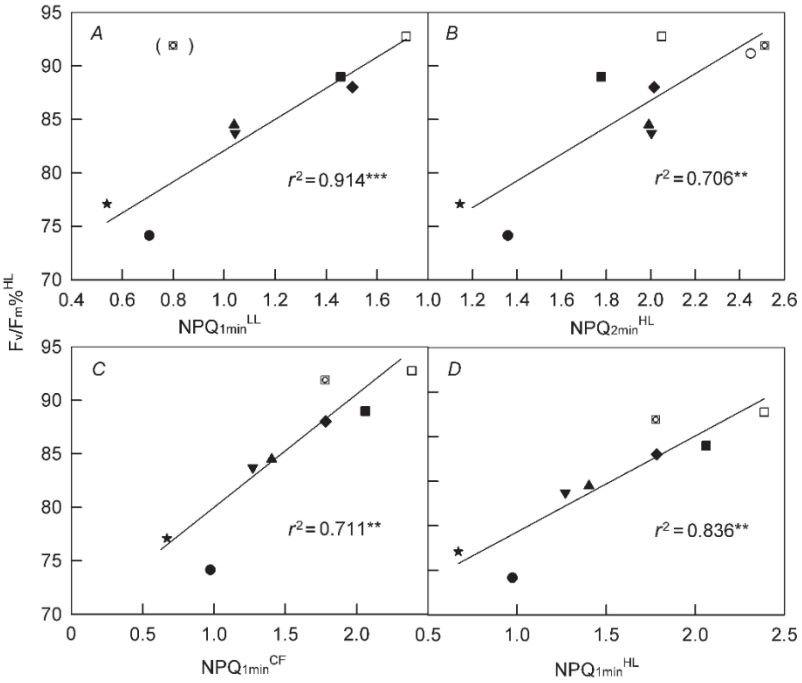

Fig. 7. Relation between nonphotochemical quenching (NPQ) values and relative value of potential quantum efficiency of PSII in leaves exposed to 2,000 μmol m^{-2} s^{-1} PPFD for 30 min [F_v/F_m%^{HL}, F_v/F_m of the same leaves before illumination set to 100%, data from Wong *et al*. 2012] in 3 woody species [*Alnus formosana* (⧇), *Ardisia crenata* (*downward triangles*), and *Ardisia cornudentata* (*upward triangles*)] and 4 fern species [*Pyrrosia lingus* (squares), *Asplenium antiquum* (*circles*), *Diplazium donianum* (*diamonds*), and *Archangiopteris somai* (*stars*)] cultivated under 100% (open symbols) and 10% (*closed symbols*) sunlight. Each point represents the mean of 3–5 (0 inlet CO_2) and 4–6 (ambient CO_2) leaves. 100% sunlight-grown *A. antiquum* was measured under 2,000 μmol m^{-2} s^{-1} PPFD only in 2010. NPQ$_{1min}^{LL}$ and NPQ$_{1min}^{CF}$, leaves were exposed to 100 μmol m^{-2} s^{-1} PPFD under ambient CO_2 and 0 inlet CO_2 concentration for 1 min, respectively. NPQ$_{1min}^{HL}$ and NPQ$_{2min}^{LL}$, leaves were exposed to 2,000 μmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration for 1 min (in 2011) and 2 min (in 2010), respectively]. **, *** – p<0.01, p=0.001, respectively. Symbol in parenthesis was excluded from the regression analysis.

state of high light induction; thus the leaves with high P_G tended to have high NPQ under such conditions (Fig. 4*A*). In contrast, 30 min after the start of HL induction, probably due to photosynthesis being activated, the leaves with high P_G tended to have low NPQ (Fig. 4*C*).

At the initial state of HL induction, the fastest-developing NPQ component is an important mechanism to protect the photosystem (Müller *et al*. 2001, Murchie and Niyogi 2011). Even if photoprotection of PSII is affected by antioxidation (Golan *et al*. 2006, Murchie and Niyogi 2011), under HL induction, the leaves with higher NPQ at the initial 1 or 2 min still tended to have a lower degree of photoinhibition at the final stage of induction (30 min) (Fig. 7*B*,*D*), with more NPQ relaxation when leaves were shifted from light to dark (Fig. 2*A* and data not shown). The initial developing NPQ values, obtained under different light and CO_2 conditions, correlated with each other (Fig. 5); thus, these NPQ values showed significant correlation with the degree of photoinhibition (Fig. 7). Because NPQ values can be quickly obtained during the initial 1 or 2 min of light induction, NPQ might be useful to assess the light-adaptation capability of species. However, at the initial 1 min of light induction under LL and ambient CO_2 concentrations, *A. formosana* showed the low NPQ due to the high P_G, which was located at far left of the NPQ-F_v/F_m% regression line (Fig. 7*A*). In contrast, under HL (Fig. 4*A*) or 0 inlet CO_2 concentrations (Fig. 3*A*), *A. formosana* showed the high NPQ at the initial stage of light induction because of high excess of light energy. Therefore, NPQ values were located near the NPQ-F_v/F_m% regression line (Fig. 7*B–D*). Therefore, NPQ should be obtained under conditions with high excess of light energy to assess the light-adaption capability.

In the present study, we found that the formation and relaxation of the transient NPQ during light induction was closely related to light intensity, light-adaption capability of species, and photosynthetic rate of leaves. Under LL, marked transient (first 1–2 min of light induction) NPQ was found only in leaves with high P_G (one pioneer tree and one mild shade–adapted fern). In leaves with low P_G and ETR, the NPQ relaxation was low or absent up to 20 min after the start of LL induction regardless of species characteristics or inhibition by low CO_2 concentration. Under high excess of light energy (HL, 2,000 μmol m^{-2} s^{-1} PPFD or 0 inlet CO_2 concentration), species adapted to or leaves acclimated to HL tended to have high NPQ at the initial 1 or 2 min, and showed low photoinhibition at the final state (30 min) of light induction. Because these fastest-developing NPQ values can be quickly and easily obtained, they might be helpful in further ecophysiological research.

References

Adams, W.W., Zarter, C.R., Ebbert, V., Demmig-Adams, B.: Photoprotective strategies of overwintering evergreens. – BioScience **54**: 41-49, 2004.

Asada, K.: The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. – Annu. Rev. Plant Phys. Plant Mol. Biol. **50**: 601-639, 1999.

Bailey, S., Horton, P., Walters, R.G.: Acclimation of *Arabidopsis thaliana* to the light environment: the relationship between photosynthetic function and chloroplast composition. – Planta **218**: 793-802, 2004.

Ballottari, M., Dall'Osto, L., Morosinotto, T., Bassi, R.: Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. – J. Biol. Chem. **282**: 8947-8958, 2007.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504. 1987.

Boardman, N.K.: Comparative photosynthesis of sun and shade plants. – Annu. Rev. Plant Phys. **28**: 355-377, 1977.

Demmig-Adams, B., Adams, W.W., Barker, D.H. *et al*.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Demmig-Adams, B., Ebbert, V., Mellman, D.L. *et al.*: Modulation of PsbS and flexible *vs*. sustained energy dissipation by light environment in different species. – Physiol. Plantarum **127**: 670-680, 2006.

Finazzi, G., Johnson, G.N., Dall'Osto, L. *et al*.: A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. – P. Natl. Acad. Sci. USA **101**: 12375-12380, 2004.

Golan, T., Müller-Moulé, P., Niyogi, K.K.: Photoprotection mutants of *Arabidopsis thaliana* acclimate to high light by increasing photosynthesis and specific antioxidants. – Plant Cell Environ. **29**: 879-887, 2006.

Kalituho, L., Beran, K.C., Jahns, P.: The transiently generated nonphotochemical quenching of excitation energy in *Arabidopsis* leaves is modulated by zeaxanthin. – Plant Physiol. **143**: 1861-1870, 2007.

Kato, M.C., Hikosaka, K., Hirotsu, N., Makino, A., Hirose, T.: The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoinactivation in photosystem II. – Plant Cell Physiol. **44**: 318-325, 2003.

Lambers, H., Chapin, F.S. III, Pons, T.L.: Plant Physiological Ecology. Pp. 25-46. Springer-Verlag, New York 1998.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence-a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Miyake, C., Okamura, M.: Cyclic electron flow within PSII protects PSII from its photoinhibition in thylakoid membranes from spinach chloroplasts. – Plant Cell Physiol. **44**: 457-462, 2003.

Müller, P., Li, X.P., Niyog, K.K.: Non-photochemical quenching: a response to excess light energy. – Plant Physiol. **125**: 1558-1566, 2001.

Murchie, E.H., Niyogi, K.K.: Manipulation of photoprotection to improve plant photosynthesis. – Plant Physiol. **155**: 86-92, 2011.

Nilkens, M., Kress, E., Lambrev, P., *et al*.: Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in *Arabidopsis*. – BBA-Bioenergetics **1797**: 466-475, 2010.

Peterson, R.B.: Regulation of electron transport in photosystems I and II in C_3, C_3-C_4, and C_4 species of *Panicum* in response to changing irradiance and O_2 levels. – Plant Physiol. **105**: 349-356, 1994.

Pearcy, R.W., Sims, D.A.: Photosynthetic acclimation to changing light environments: Scaling from the leaf to the whole plant. – In: Caldwell, M.M., Pearcy, R.W. (ed): Exploitation of Environmental Heterogeneity by Plants: Ecophysiological Processes Above- and Belowground. Pp. 145-174. Academic Press, San Diego 1994.

Robinson, J.M.: Nitrite photoreduction *in vivo* is inhibited by oxygen. – Plant Physiol. **92**: 862-865, 1990.

Scholes, J.D., Press, M.C., Zipperlen, S.W.: Differences in light energy utilisation and dissipation between dipterocarp rain forest tree seedlings. – Oecologia **109**: 41-48, 1997.

Thaipratum, R., Melis, A., Svasti, J., Yokthongwattana, K.: Analysis of non-photochemical energy dissipating processes in wild type *Dunaliella salina* (green algae) and in zea1, a mutant constitutively accumulating zeaxanthin. – J. Plant Res. **122**: 465-476, 2009.

Wong, S.L., Chen, C.W., Huang, H.W., Weng, J.H.: Using combined measurements for comparison of light induction of stomatal conductance, electron transport rate and CO_2 fixation in woody and fern species adapted to different light regimes. – Tree Physiol. **32**: 535-544, 2012.

Yamazaki, J.Y., Yoda, E., Takahashi, A., Sonoike, K., Maruta, E.: Pacific Ocean and Japan Sea ecotypes of Japanese beech (*Fagus crenata*) differ in photosystem responses to continuous high light. – Tree Physiol. **27**: 961-968, 2007.

Zulfugarov, I.S., Ham, O.K., Mishra, S.R. *et al*.: Dependence of reaction center-type energy-dependent quenching on photosystem II antenna size. – BBA-Bioenergetics **1767**: 773-780, 2007.