Photoinhibition and xanthophyll cycle activity in bayberry (*Myrica rubra*) leaves induced by high irradiance

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Abstract

The effect of high irradiance (HI, photosynthetically active photon flux density of 1 300 μmol m^{-2} s^{-1}) on net photosynthetic rate (P_N), chlorophyll fluorescence parameters, and xanthophyll cycle components were studied in fruit tree bayberry leaves. HI induced the photoinhibition and inactivation of photosystem 2 (PS2) reaction centres (RCs), which was characterized by decreased P_N, maximum yield of fluorescence after dark adaptation (F_m), photochemical efficiency of PS2 (F_v/F_m) and quantum yield of PS2 (Φ_{PS2}), and increased reduction state of Q_A ($1 - q_P$) and non-photochemical quenching (NPQ). Initial fluorescence (F_0) showed a decrease after the first 2 h, and subsequently increased from the third hour exposure to HI. Furthermore, a greater increase in the ratio $(F_i - F_0)/(F_p - F_0)$ which is an expression of the proportion of the Q_B non-reducing PS2 centres, whereas a remarked decrease in the slope of F_i to F_p which represents the rate of Q_A reduction was observed in leaves after HI exposure. Additionally, HI caused an increase in the pool size of the xanthophyll cycle pigments and sustained elevated contents of zeaxanthin (Z), antheraxanthin (A), and de-epoxidation state (DES) at the end of the irradiation period. During HI, decreased F_m, F_v/F_m, Φ_{PS2}, NPQ, slope of F_i to F_p, V+A+Z, and DES, and increased F_0, $1 - q_P$, ratio $(F_i - F_0)/(F_p - F_0)$, and V were observed in dithiothreitol (DTT)-fed leaves compared to control ones under the same conditions. Hence photoinhibition caused by HI in bayberry was probably attributed to inactivation of PS2 RCs, and photoprotection from photodamage were mainly related to the xanthophyll cycle-dependent heat dissipation in excess photons.

Additional key words: chlorophyll fluorescence; dithiothreitol; intercellular CO_2 concentration; net photosynthetic rate; photosystem 2 reaction centres.

Introduction

Photons are the ultimate energy source for photosynthesis, their deficit inevitably limits photosynthesis. However, exposure of plants to a high irradiance (HI) can cause a depression of photosynthesis and photosystem 2 (PS2) efficiency, and even photo-damage due to the absorbed photon energy that is transferred to the reaction centres (RCs) exceeds the utilization (Long *et al*. 1994). This decrease in photosynthetic rate induced by HI is called photoinhibition. The phenomenon has been studied for over 100 years using a large variety of biochemical, physiological, biophysical, and genetic methods (Adir *et al*. 2003). Although many documents on the subject have been published in different plant species, the mechanism of photoinhibition is still obscure. The existence of species-specific differences in photosynthetic response to various irradiances results in a lack of general evidence as to an integration of photo-damage, repair processes, and down-regulation of photosynthesis caused by HI (Chen *et al*. 2003).

There are different protective mechanisms of photo-

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Abbreviations: A, antheraxanthin; C_a, ambient CO_2 concentration; C_i, intercellular CO_2 concentration; Chl, chlorophyll; DES, de-epoxidation state of the xanthophyll cycle pigments; DTT, dithiothreitol; F_m, maximum yield of fluorescence after dark adaptation; F_0, initial fluorescence; F_v/F_m, maximum photochemical efficiency of PS2 with all reaction centres open; NPQ, non-photochemical quenching; PFD, photon flux density; P_N, net photosynthetic rate; PS2, photosystem 2; RC, reaction centre; V, violaxanthin; Z, zeaxanthin; $1 - q_P$, reduction state of Q_A; Φ_{PS2}, quantum yield of PS2.
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synthetic apparatus from photoinhibition. Mechanisms such as non-photochemical thermal dissipation of excess energy (Demmig-Adams and Adams 1996), short and long-term dynamic regulation of the antenna size (Aro *et al*. 1993), and cyclic electron flow within PS2 (Miyake and Okamura 2003) serve to protect the photosynthetic apparatus. Other mechanisms for protection involve processes such as chlorophyll (Chl) content change (Murchie and Horton 1997), chloroplast movement, increase in the capacity for scavenging the active oxygen species by means of increase in scavenging enzyme activity and/or concentration of non-enzymatic antioxidants (Foyer *et al*. 1994), and leaf movement.

Photoinhibition was not only attributed to degradation of the D1 protein (Rintamaki *et al*. 1995, Guo *et al*. 1996), but also to an increase in the rate of thermal energy dissipation (Aro *et al*. 1993, Guo *et al*. 1999). The xanthophyll cycle plays a key role in photo-protection of plants exposed to HI stress (Krause *et al*. 1995, Laisk and Oja 2000, Song *et al*. 2003). Although the exact mechanisms of energy dissipation are not well understood, strong evidence supports the hypothesis that zeaxanthin (Z) formation, *via* the operation of the xanthophyll cycle, is responsible for the majority of dissipation of the excess excitation energy (Demmig-Adams and Adams 1992, Pandey *et al*. 2004) and can be detected by measuring Chl fluorescence (as non-photochemical quenching, NPQ).

Since Chl fluorescence has been widely used as effective parameter reflecting the physiological aspects of photosynthetic process in plants (Hong and Xu 1999), it is used to estimate the extent of photoinhibition (Ögren 1991). Much evidence over the past decade has shown that photoinhibition is often related to inhibition of PS2 photochemistry, which is accompanied with a decrease in F_v/F_m and F_m (Krause and Weis 1991, Guo *et al*. 1999), and an increase in F_0 in several plant species (Hong and Xu 1999). In a research on the pathway for photo-inactivation of PS2 function, Vass *et al*. (1992) concluded that the increase in F_0 can be due to the formation of double reduction and possible protonation of Q_A. Moreover, an increase in F_0 has been attributed to the disconnection of PS2 RCs (Srivastava *et al*. 1997).

Bayberry (*Myrica rubra*), an important evergreen fruit tree, is frequently cultivated in hillsides in southern China (Kang *et al*. 2002). A cultivation of bayberry together with deciduous trees is generally recommended for the protection of ecological systems for soil and manure (Wang *et al*. 2001). This cultivation practice is also able to produce high quality plants and fruits because it presumably reduces foliar damage caused by HI that occurs during the summer, which may impair their field performance (Liu 1998). Bayberry is thought to be a shade adapted plant, in which the inhibitory effect of HI on photosynthesis in the fruit tree is still unknown. An increase in non-photochemical quenching (NPQ) may be important for the protection of the photosynthetic apparatus (Demmig-Adams 1990, Demmig-Adams *et al*. 1996). Therefore, it is needed to understand how HI influences its photosynthesis by measuring the Chl fluorescence parameters, finding how NPQ changes, and estimate its relation to photo-protection in bayberry. A study on the influence of HI on the xanthophyll cycle in this fruit tree is also needed.

Materials and methods

Plants: Bayberry (*Myrica rubra*) plants were grown in pots in a phytotron at 25/20 °C (day/night). The photon flux density (PFD) of six dysprosium lamps was about 700 μmol m^{-2} s^{-1} at the top of the plant during a 12/12 h (light/dark) period. The relative humidity was about 60 %. The plants were irrigated daily.

Gas exchange: The potted plants were transferred from the phytotron to the photosynthesis laboratory. Net photosynthetic rate (P_N) and intercellular CO_2 concentration (C_i) were measured in fully developed leaves in plant using an open system (*HCM-1000*, *H. Walz*, Effeltrich, Germany) under artificial light source (four 400 W dysprosium lamps above 8-cm water layer serving as heat filter). Measurements of irradiance response of photosynthesis were performed at 25 °C, with irradiance from approximately 0 to 1 200 μmol m^{-2} s^{-1} on the surface of measured leaves.

HI stress: The potted plants were transferred from the phytotron to the photosynthesis laboratory. The fully developed leaves in plant were irradiated for 0, 1, 2, 3, and 4 h at PFD of 1 300 μmol m^{-2} s^{-1} (stronger than the irradiance saturating photosynthesis in leaves of plants grown in the phytotron), respectively.

Chl fluorescence of leaves was measured at room temperature (25 °C) with a portable fluorometer (*PAM-2000*, *H. Walz*, Effeltrich, Germany) after the leaves were dark-adapted for 20 min (Guo *et al*. 2005). The fluorometer was connected to a trifurcated fibre optic (*2010-F*) and to a computer with data acquisition software (*PAMWin 1.03*). The minimal fluorescence (F_0) with all PS2 RCs open was measured with modulated radiation which was sufficiently low (<0.1 μmol m^{-2} s^{-1}) not to induce significant variable fluorescence. The maximal fluorescence (F_m) with all PS2 RCs closed was determined by a 0.8-s saturating pulse at 8 000 μmol m^{-2} s^{-1} in dark-adapted leaves. Then, the leaves were continuously irradiated with 336 μmol m^{-2} s^{-1} of "white actinic light". The steady state value of fluorescence (F_s) was thereafter recorded and a second saturating pulse at 8 000 μmol m^{-2} s^{-1} was imposed to determine maximal fluorescence in the light-adapted state (F_m'). F_0' was basal fluorescence

after 5 μmol m^{-2} s^{-1} of far-red irradiation. The reduction state of Q_A $1 - q_P = 1 - (F_m' - F_s)/(F_m' - F_o')$, non-photochemical quenching $NPQ = (F_m - F_m')/F_m'$, and the actual quantum yield of PS2 photochemistry $\Phi_{PS2} = (F_m' - F_s)/F_m'$ were calculated as defined by Genty *et al*. (1989).

The poly-phasic rise of fluorescence transients (OIP) was measured in dark-adapted leaves suddenly irradiated with "white light" of 491 μmol m^{-2} s^{-1} at a sampling rate of 1 000 μs $point^{-1}$ (Govindjee 1995, Guo *et al*. 2004).

Xanthophyll analysis: Immediately after Chl fluorescence measurements, one 1.54 cm^2 leaf disc was punched from the leaf and frozen in liquid nitrogen. Frozen leaf discs were stored at –80 °C until analysis. To extract pigments, a leaf disc was pulverized in liquid nitrogen in a mortar, followed by grinding in 1.5 cm^3 cold 80 % acetone. The extract was centrifuged at 10 000×*g* for 3 min. The supernatant was passed through a 0.45 μm membrane filter before HPLC injection. Photosynthetic pigments were separated and quantified by HPLC (*Waters-510 B*) following the protocol of Eskling *et al.* (1997). De-epoxidation state (DES) was calculated as (Z+A)/(Z+A+V), where A is antheraxanthin, V violaxanthin, and Z zeaxanthin.

DTT feeding: Leaves were cut at the end of the leaf stalk and placed in vials containing 5 mM DTT or water for the control. Then, the leaves were irradiated by 20 μmol m^{-2} s^{-1} at 25 °C for 8 h.

Statistical analysis: Results were subjected to analysis of variance (ANOVA) using *SPSS 8.0*' software and mean values were compared by the Tukey's test (p=0.05).

Results

Saturation irradiance for P_N in *M. rubra* leaves was at about 700 μmol m^{-2} s^{-1} (Fig. 1). HI (1 300 μmol m^{-2} s^{-1}) caused an inhibition of photosynthesis (Fig. 2). A decline in P_N was observed in plants after 1 h of HI treatment. With prolongation of HI treatment until 2 h, there was an abrupt decrease in P_N by about 46 % compared with that at the end of first hour of HI treatment. Later, P_N decreased gradually with the prolonged HI. In contrast, there was a progressive increase in C_i/C_a (intercellular CO_2 concentration/ambient CO_2 concentration) over the course of HI treatment (Fig. 2). As shown in Fig. 2, P_N and C_i/C_a in HI treated leaves recovered at dark for nearly 17 h as well.

The changes in Chl fluorescence parameters measured in *M. rubra* leaves during HI treatment for 4 h and subsequent dark recovery for 17 h are shown in Fig. 3. During HI treatment, there was a gradual decline in F_0 within the first two hours, the value slowly declining over the two-hour period by 17 % prior to treatment, followed by a rise after another two-hour period, the F_0 reaching even higher than the initial value before HI treatment. Relaxation of F_0 was observed 4 h after the dark treatment, although F_0 maintained slightly higher than the initial value before HI treatment even for 17 h (Fig. 3*A*). A considerably sharp drop in F_m value by about 50 % was observed during HI treatment. The recovery of F_m was rapid after first 4 h after dark treatment, and almost complete after 17 h (Fig. 3*B*). $1 - q_P$ (reduction state of Q_A) and NPQ increased rapidly to extremely high values, when the behaviours of F_v/F_m and Φ_{PS2} were similar to that of F_m during HI treatment and the subsequent dark recovery (Figs. 3*C–F*).

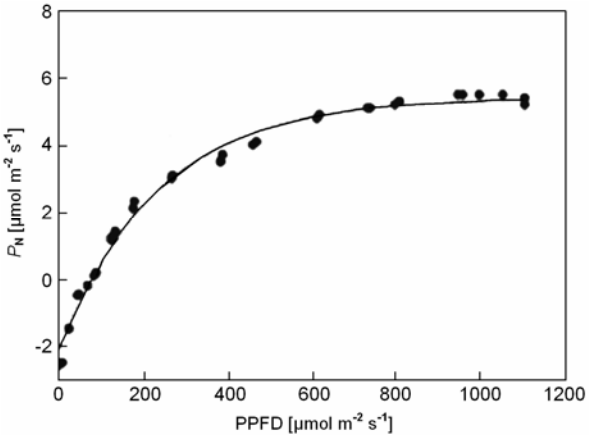

Fig. 1. Irradiance (PFD) response curve of photosynthesis in *Myrica rubra* leaves.

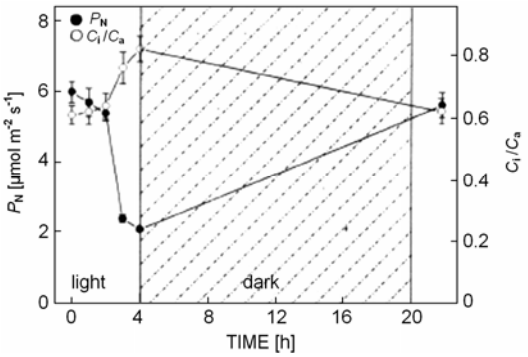

Fig. 2. Changes of net photosynthetic rate (P_N) and ratio of intercellular CO_2 concentration (C_i) to ambient CO_2 concentration (C_a) in *Myrica rubra* leaves under high irradiance of 1 300 mol m^{-2} s^{-1}. Means of 6 replicates ± SE.

The ratio $(F_i - F_0)/(F_p - F_0)$, which represents the percentage of those Q_B-non-reducing PS2 RCs, continuously increased over the duration of HI exposure (Fig. 4*A*). The slope from F_i to F_p, which is indicative of the rate of Q_A reduction, however, decreased with the increase in the duration of exposure to HI (Fig. 4*B*). In the subsequent dark recovery, $(F_i - F_0)/(F_p - F_0)$ and the

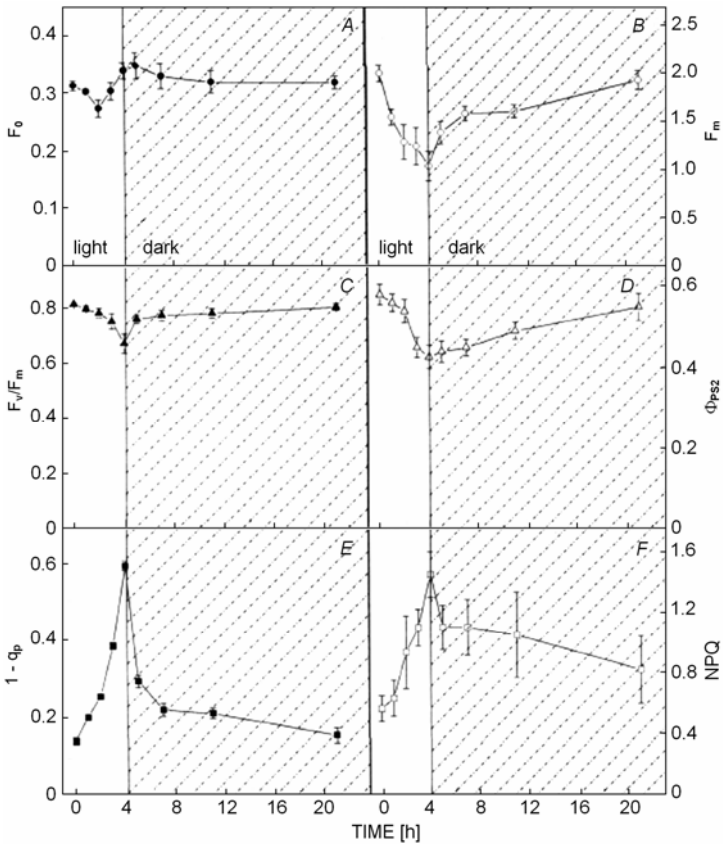

Fig. 3. Development of (*A*) initial fluorescence (F_0), (*B*) maximal fluorescence (F_m), (*C*) photosystem 2 (PS2) photochemical efficiency (F_v/F_m), (*D*) quantum yield of PS2 (Φ_{PS2}), (*E*) reduction state of Q_A ($1 - q_P$), and (*F*) non-photochemical quenching (NPQ) in *Myrica rubra* leaves under high irradiance (1 300 μmol m^{-2} s^{-1} PFD) and dark recovery. Means of 6 replicates ± SE.

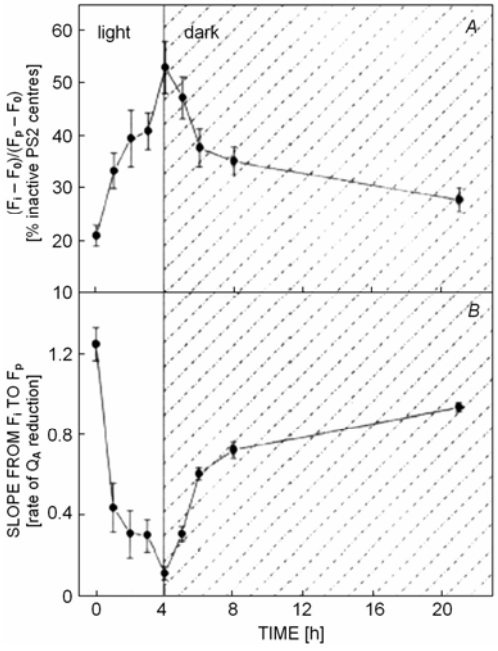

Fig. 4. Changes of (*A*) $(F_i - F_0)/(F_p - F_0)$ [% inactive PS2 centres]) and (*B*) slope from F_i to F_p (rate of Q_A reduction) in *Myrica rubra* leaves during high irradiance (1 300 μmol m^{-2} s^{-1}) treatment and dark recovery. Means of 6 replicates ± SE.

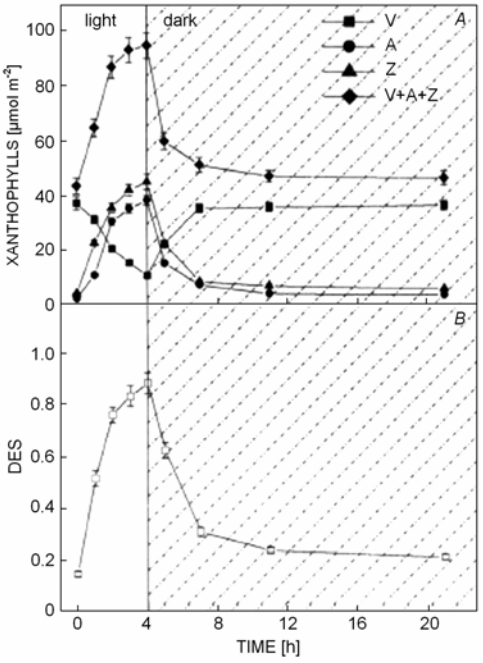

Fig. 5. Changes of (*A*) xanthophyll cycle pigments (antheraxanthin A, violaxanthin V, and zeaxanthin Z) and (*B*) de-epoxidation state of the xanthophyll cycle pigments (DES) in *Myrica rubra* leaves during high irradiance (1 300 μmol m^{-2} s^{-1}) and dark recovery. Means of 6 replicates ± SE.

slope from F_i to F_p recovered, the rate of recovery being much slower during the late period of dark treatment. Both of them did not reach the initial value at the end of dark recovery for 17 h (Fig. 4).

HI for 4 h led to a decline in V content by about 70 % and an elevation of about 16-fold, 10-fold, and 1.2-fold in A, Z, and xanthophyll cycle pool (A+Z+V), respectively (Fig. 5*A*). In the present experiment, the de-epoxidation state of the xanthophyll cycle (DES) also increased under HI (Fig. 5*B*), and it could largely recover after 4 h in the dark.

The effect of dithiothreitol (DTT) treatment on the Chl fluorescence parameters during photoinhibition is shown in Table 1. In this case F_0, $1 - q_P$, $(F_i - F_0)/(F_p - F_0)$, and slope from F_i to F_p were significantly enhanced, while F_m, F_v/F_m, Φ_{PS2}, and NPQ were significantly reduced by 5 mM DTT. Effects of DTT on changes in xanthophyll contents and the DES of the xanthophyll cycle in *M. rubra* leaves under HI for 2 h are shown in Table 2. During HI, A+Z decreased markedly in DTT-fed leaves relative to that of the control. Similarly, the de-epoxidized ratio of the xanthophyll cycle, (A+Z)/(V+A+Z), also decreased. On the contrary, V increased in DTT-fed leaves compared with the control ones.

A close relationship between $1 - q_P$ or NPQ and DES was obtained for the combined data in HI-leaves. $1 - q_P$ and NPQ could be fitted rather well to a curvilinear fashion, and increased as a function of the ratio of A+Z to V+A+Z (Fig. 6).

A linear regression statistics showed that linear correlation was obtained between increasing $1 - q_P$ and $(F_i - F_0)/(F_p - F_0)$ [$y_{(Fi-F0)/(Fp-F0)} = 17.38 + 68.70\ x_{1-qP}$ ($r = 0.882$, $p<0.01$)] (Fig. 7).

Table 1. Effect of dithiothreitol (DTT) on chlorophyll fluorescence parameters in *Myrica rubra* leaves after high irradiance (1 300 μmol m^{-2} s^{-1}) for 2 h. Means of 6 replicates ± SE. *Values marked with different letters* are significantly different according to Tukey's test ($p<0.05$).

Fluorescence parameters	Treatment	
	–DTT	+DTT (5 M)
F_0	0.274±0.015 b	0.424±0.008 a
F_m	1.251±0.066 a	0.811±0.021 b
F_v/F_m	0.781±0.014 a	0.477±0.036 b
Φ_{PS2}	0.517±0.022 a	0.425±0.047 b
$1 - q_P$	0.266±0.009 b	0.424±0.008 a
NPQ	0.934±0.006 a	0.461±0.008 b
$(F_i - F_0)/(F_p - F_0)$	0.385±0.027 b	0.633±0.041 a
Slope from F_i to F_p	0.305±0.018 a	0.156±0.032 b

Discussion

The inactivation of PS2 RCs: Photoinhibition in plants occurs when plants experience irradiance at or above the level saturating for growth (Guo *et al*. 1999, Adir *et al*. 2003). We found that the saturating irradiance of P_N in *M. rubra* leaves was at about 700 μmol m^{-2} s^{-1} (Fig. 1). Thus photoinhibition induced by HI of 1 300 μmol m^{-2} s^{-1} was used in subsequent measurements. P_N decreased after 1 h of HI and continued to drop until the end of HI treatment. Meanwhile, a gradual increase in C_i/C_a was observed during the duration of HI (Fig. 2), suggesting that photoinhibion in *M. rubra* leaves was probably not mainly due to stomatal limitation, which may lead to a decrease in P_N in some cases.

In the present study, photoinhibition was apparently reversible, at least in part if not completely, although irreversible photoinhibition was evident after a prolonged exposure to HI (Hideg and Murata 1997). This is further confirmed by the results of subsequent dark recovery: leaves reached maximum PS2 photochemistry efficiency and the rate of recovery that agrees with the report of Krause *et al*. (1995).

Table 2. Effect of dithiothreitol (DTT) on xanthophylls in *Myrica rubra* leaves after high irradiance (1 300 μmol m^{-2} s^{-1}) for 2 h. Means of 6 replicates ± SE. *Values marked with different letters* are significantly different according to Tukey's test ($p<0.05$). For abbreviations see the text.

Treatment	Xanthophylls [μmol m^{-2}]			
	V	A+Z	V+A+Z	DES
Control	20.54±1.32 b	66.38±3.57 a	86.92±4.15 a	0.764±0.034 a
+DTT (5 M)	37.36±2.11 a	31.49±1.23 b	69.86±3.66 b	0.451±0.026 b

The reduction in F_v/F_m, resulting from increased F_0 and/or decreased F_m, is a convenient measure of photoinhibition and an indicator of reduction in potential PS2 photochemical efficiency, since the F_v/F_m ratio is closely correlated with the quantum yield of photosynthetic O_2 evolution or CO_2 assimilation (Harel *et al*. 2004). An increase in F_0 may be induced by the inactivation of part of PS2 RCs (Hong and Xu 1999). In mature grape leaves, when F_0 increased under HI, some PS2 RCs lost their photochemical activity, as indicated by a marked decline in the photochemical efficiency of PS2 (F_v/F_m) (Bertamini and Nedunchezhian 2003).

Photoinhibition can be reflected by Chl fluorescence, although F_0 may have a distinct pattern after HI in various species (Demmig-Adams and Adams 1992, Hong

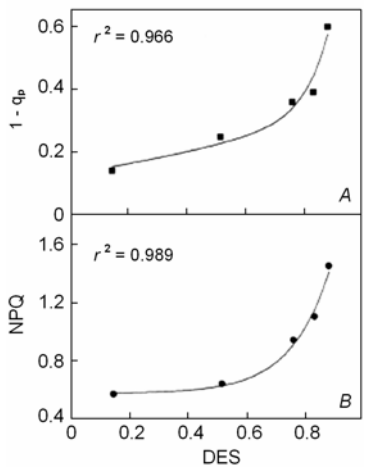

Fig. 6. Reduction state of (*A*) Q_A ($1 - q_P$) and (*B*) non-photochemical quenching (NPQ) in relation to de-epoxidation state of the xanthophyll cycle pigments (DES) in *Myrica rubra* leaves under high irradiance (1 300 μmol m^{-2} s^{-1} PFD). Relativity: for $1 - q_P$, $r^2 = 0.966$, $p < 0.01$; for NPQ, $r^2 = 0.989$, $p < 0.01$.

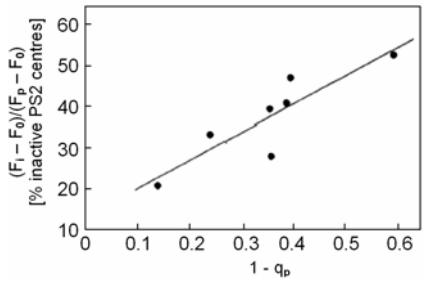

Fig. 7. $(F_i - F_0)/(F_p - F_0)$ [% inactive PS2 centres] in relation to reduction state of Q_A ($1 - q_P$) in *Myrica rubra* leaves under high irradiance (1 300 μmol m^{-2} s^{-1} PFD). Regression equation: $y = 17.38 + 68.70\ x$ ($r = 0.882$, $p < 0.01$).

and Xu 1999), reflecting different major protective mechanism against photo-damage (Hong and Xu 1999): the xanthophyll cycle-dependent non-radiative energy dissipation or inactivation of some PS2 RCs. The xanthophyll cycle-dependent non-radiative energy dissipation may play a central role in plants, in which the F_0 remained unchanged or decreased when exposed to HI, while the latter occurred in plants showing an increased F_0 during photoinhibition. However, in this study, F_0 pattern showed a unique characteristic in *M. rubra* leaves during photoinhibition, it decreased during the exposure of first two hours to HI and then decreased with a prolonged HI treatment (Fig. 3). Therefore, the mechanisms underlying the processes of photo-protection might include a complex step: the xanthophyll cycle-dependent heat dissipation was the main protective mechanism at early stage of HI exposure, and this was further confirmed by the experiment on xanthophyll cycle pool size and DTT treatment. After 2 h of DTT treatment, the F_0 increased, while other Chl fluorescence parameters decreased (Table 1). A decrease in F_v/F_m resulting solely from damage of PS2 RCs would be expected to be accompanied by an increase in F_0, whereas an increase in non-radiative energy dissipation would lead to both F_0 and F_m quenching (Vavilin *et al*. 1995). This finding together with the experiment on the DTT treatment suggests that the lowered F_v/F_m arose in *M. rubra* leaves mainly by non-radiative energy dissipation.

However, increase in F_0 was found 3 h after HI (Fig. 3*A*). Since the xanthophyll cycle-dependent heat dissipation always lead to a decreased F_0 under HI (Demmig-Adams and Adams 1996), the increase in F_0 is likely not to have this protective mechanism. Thus increased F_0 might be attributed to inactivation of PS2 RCs (Kornyeyev *et al*. 2003).

The fluorescence parameter F_v/F_m is a good measure of photoinhibition, and a decrease in F_v/F_m under photoinhibitory conditions is often attributed to the loss of D1 protein (Estelle 2001). If the photoinhibition in bayberry is involved in degradation of D1 protein needs to be further examined.

The rapid fluorescence induction kinetics was assessed to determine whether HI induced the changes in the heterogeneity of PS2 RCs. During photoinhibition in bayberry, there were an increase in $1 - q_P$, reduction state of Q_A, q_P, and the ratio $(F_i - F_0)/(F_p - F_0)$ and a decrease in the slope from F_i to F_p, indicating that HI affected the proportion of Q_B-non-reducing PS2 RCs and Q_A reduction (Figs. 3*E* and 4). Reduction of Q_A to Q_A^- is associated with the primary photochemical reactions of PS2 (Strasser *et al*. 1995). In this experiment, a greater increase in the ratio $(F_i - F_0)/(F_p - F_0)$ was observed during HI, suggesting that photoinhibition was accompanied with an inactivation of PS2 RCs. Meanwhile, the decrease in the rate of Q_A reduction occurred during photoinhibition, implying that the reducing activity of PS2 was retarded by HI. The results gave more evidence that environmental stress in plants may cause changes in rapid Chl fluorescence kinetics reflecting the state of PS2 (Lu *et al*. 2003).

The roles of NPQ and xanthophyll cycle: According to Demmig-Adams (1990), the irradiance resulting in an increase in $1 - q_P$ to a value above 0.6 is excess irradiance. Therefore the *M. rubra* plants under excess excitation pressure had $1 - q_P$ close to 0.6 after spending 4 h in HI (Fig. 3). $1 - q_P$ is a measure of the redox state of Q_A and thus reflects the balance between photon energy absorbed through the photochemical reactions of PS2 and the energy utilized through the reactions of electron transport and CO_2 fixation. Therefore, PS2 excitation pressure may be elevated by increasing irradiance. Although debates on $1 - q_P$ as a indicator of the relative redox state of PS2 RCs exist (Campbell *et al.* 1998), we maintain that $1 - q_P$ is a reasonable indicator of PS2 closure in *M. rubra* plants exposed to HI (Figs. 6 and 7).

Non-photochemical quenching (NPQ) is also a parameter reflecting photo-protective dissipation of excess absorbed energy as heat, when electron flow to the Calvin

cycle becomes slowed (Demmig-Adams 1990). It is a good indicator of the concentration of energy dissipating complexes. We observed that NPQ increased considerably after HI exposure (Fig. 3*F*), indicating that photoprotection requires not only the physical presence of de-epoxidized xanthophylls but also NPQ (Xu *et al*. 2004).

The xanthophyll cycle is important in photoprotection (Müller *et al.* 2001). When exposed to continuous HI, the excess excitation of PS2 generates a high ΔpH across the thylakoid membrane and activates the inter-conversion in the xanthophyll cycle resulting in a relative increase in Z content on expense of V. The xanthophyll cycle pool size in *M. rubra* leaves was increased during HI (Fig. 5). Similar results were obtained in soybean and barley plants grown under a high PFD (Pandey *et al*. 2004, Špundová *et al*. 2005). The xanthophyll pool was effective in photo-protection of *M. rubra* leaves under photoinhibition. Under stress, pear leaves also showed a significant increase in the xanthophyll cycle pool (Morales *et al*. 1994). This rise in the ratio of xanthophyll cycle pool indicates that, in addition to downsizing light-harvesting Chl antennae, increasing the relative concentration of xanthophylls in the light-harvesting complexes of PS2 was employed by leaves to cope with HI.

Close correlations have been found between Z or Z+A contents and NPQ in different plant species under a wide range of environmental conditions (Demmig-Adams and Adams 1996). Inhibition of V de-epoxidase activity by DTT resulted in a decrease in NPQ (Table 1). Characterization of the *Arabidopsis* mutant npq1 (Niyogi *et al*. 1998) and antisense tobacco plants (Chang *et al*. 2000, Sun *et al*. 2001), both of which had little V de-epoxidase activity, showed that NPQ was greatly suppressed under high PFD, which confirmed that Z is necessary for most NPQ. In this context, our finding that NPQ was highly correlated with DES on a xanthophyll cycle pool basis (Fig. 6) indicates that the increased thermal dissipation of excitation energy is dependent on the enhanced xanthophyll cycle activity.

V de-epoxidase, which catalyses the conversion of V to Z *via* A, is localized in the thylakoid lumen and is activated by low lumen pH upon exposure to HI (Eskling *et al*. 1997). Low lumen pH also results in protonation of proteins in the PS2 light-harvesting complexes. The interaction of Z and/or A with the specific sites of the protonated PS2 proteins induces a conformational change that leads to increased quenching of excitation energy (Müller *et al*. 2001). Although no attempt was made to determine the response of trans-thylakoid pH to irradiance in this experiment, the close correlation between DES and NPQ (Figs. 5 and 6) suggests that changes in trans-thylakoid pH may be highly coordinated with Z+A formation. These results indicated that DES may play a role in thermal dissipation, but the exact contribution of DES to thermal dissipation remains to be determined.

In conclusion, PS2 photochemical efficiency decline is due to inactive PS2 RCs in *M. rubra* leaves during HI and its subsequent recovery upon return to darkness. Both xanthophyll cycle pool size and conversion of V to Z under HI were enhanced. The increased thermal dissipation of excess absorbed photons in *M. rubra* leaves under HI was closely related to their elevated DES levels.

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