

REVIEW

Non-radiative dissipation of absorbed excitation energy within photosynthetic apparatus of higher plants

M. ŠTROCH*, V. ŠPUNDA^{*,**,***}, and I. KURASOVÁ*

*Department of Physics, Faculty of Science, Ostrava University, 30. dubna 22, 701 03 Ostrava 1, Czech Republic**
*Institute of Physical Biology, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic***

Abstract

The review deals with thermal dissipation of absorbed excitation energy within pigment-protein complexes of thylakoid membranes in higher plants. We focus on the de-excitation regulatory processes within photosystem 2 (PS2) that can be monitored as non-photochemical quenching of chlorophyll (Chl) *a* fluorescence consisting of three components known as energy-dependent quenching (q_E), state-transition quenching (q_T), and photoinhibitory quenching (q_I). We summarize the role of thylakoid lumen pH, xanthophylls, and PS2 proteins in q_E mechanism. Further, both the similarity between q_E and q_I and specific features of q_I are described. The other routes of thermal energy dissipation are also mentioned, that is dissipation within photosystem 1 and dissipation through the triplet Chl pathway. The significance of the individual de-excitation processes in protection against photo-oxidative damage to the photosynthetic apparatus under excess photon supply is stretched.

Additional key words: carotenoids; non-photochemical fluorescence quenching; non-radiative dissipation; photoinhibition; photo-protection; photosynthesis; xanthophyll cycle.

Introduction

Higher plants in the natural environment are subject to fluctuations in irradiance on a time-scale ranging from seconds to seasons and must cope with the quantity of the photons varying over several orders of magnitude. These fluctuations together with various environmental stresses such as chilling, high temperature, drought, or nutrient deficiency result in absorption of photon energy that often exceeds the capacity for utilization of excitation energy through photosynthetic electron transport. Absorption of excess photon energy can lead to photo-oxidative damage to the thylakoid membranes due to the formation of reactive oxygen species which may interact with pigments, proteins, and lipids (Aro *et al.* 1993, Sonoike 1996). Under these circumstances, the ability to

regulate the efficiency of absorption, transfer, and utilization of excitation energy in photochemical reactions is necessary to balance the absorption and utilization of photon energy, thereby minimizing the photo-oxidative damage.

Some plants are able to adjust their capacity for photon harvesting through movement of leaves, cells, or chloroplasts or through changes in leaf reflectance (Björkman and Demmig-Adams 1994, Barnes and Cardoso-Vilhena 1996, Wada *et al.* 2003). Under long-term exposure to excessive irradiation plants adjust the composition and function of the photosynthetic apparatus in order to decrease photon absorption and increase their utilization. The typical responses are reduction of the

Received 29 March 2004, accepted 15 July 2004.

***Corresponding author; fax: +420 59 612 04 78, e-mail: Vladimír.Spunda@osu.cz

Abbreviations: A – antheraxanthin; Chl – chlorophyll; $^1\text{Chl}^*$, $^3\text{Chl}^*$ – singlet and triplet excited state of chlorophyll, respectively; CP26, CP29 – minor chlorophyll-binding proteins of light-harvesting complexes of photosystem 2; D1, D2 – reaction-centre core proteins of photosystem 2; HI – high irradiance; L – lutein; LHC2 – light-harvesting chlorophyll *a/b*-binding protein complex of photosystem 2; NPQ – non-photochemical quenching of chlorophyll *a* fluorescence; $^1\text{O}_2^*$ – singlet excited state of oxygen; P680 – primary electron donor of photosystem 2; Pheo – pheophytin; PS1, PS2 – photosystem 1 and 2, respectively; PsbS – photosystem 2 subunit encoded by the nuclear gene *PsbS*; Q_A – primary quinone electron acceptor of photosystem 2; Q_B – secondary quinone electron acceptor of photosystem 2; q_E – energy-dependent quenching; q_I – photoinhibitory quenching; RCs – reaction centres; V – violaxanthin; Z – zeaxanthin; ΔpH – trans-thylakoid pH gradient.

Acknowledgement: This work was supported by the project “Mechanism, Ecophysiology and Biotechnology of Photosynthesis” (LN00A141) of the Ministry of Education of the Czech Republic.

light-harvesting antenna size and increase in photosynthetic capacity (Melis 1998, Bailey *et al.* 2001, Kurasová *et al.* 2002). Alternative electron transport pathways can help to remove excess absorbed energy from the photosynthetic apparatus. Photorespiration (Wingler *et al.* 2000), photoreduction of oxygen by electrons on the acceptor side of photosystem (PS) 1 in the water-water cycle (Asada 2000, Ort and Baker 2002), cyclic electron transports around PS2 (Miyake and Okamura 2003) and PS1 (Munekage *et al.* 2002), and chlororespiration (Niyogi 2000, Peltier and Cournac 2002) represent such pathways. Further, numerous antioxidant molecules and scavenging enzymes are present to deal with the inevitable generation of reactive molecules (Noctor and Foyer 1998, Asada 1999, Niyogi 1999, Munné-Bosch and Alegre 2002). Repair processes for lipid peroxidation (Baier and Dietz 1999) and damaged PS2 reaction centres (RCs) (Andersson and Aro 2001) represent the last line of defence preventing progressive photo-damage of the photosynthetic apparatus.

This review deals with other mechanisms that are important for the protection of the photosynthetic apparatus against photo-oxidative damage, namely non-radiative dissipation of absorbed excitation energy. Excess excitation energy has the potential to decay *via* pathways leading to dangerous singlet oxygen formed from interaction

between ground-state triplet oxygen and triplet chlorophyll (Chl). Safe dissipation of excitation energy in the form of heat is accomplished by carotenoids which are able to quench both triplet Chl and singlet oxygen (Demmig-Adams *et al.* 1996b, Baroli and Niyogi 2000, Choudhury and Behera 2001). The major thermal dissipation process is, however, direct de-excitation of singlet excited Chl, which prevents de-excitation *via* the undesirable triplet Chl pathway. Thermal energy dissipation within PS2 can be readily monitored as non-photochemical quenching of Chl *a* fluorescence (Roháček and Barták 1999, Maxwell and Johnson 2000). In addition to non-photochemical fluorescence quenching reflecting primarily dissipative processes within PS2, thermal energy dissipation within PS1 will be also discussed. In contrast to PS2, closure of PS1 RCs does not induce variable Chl fluorescence and therefore the estimation of thermal energy dissipation within PS1 requires some other methods such as photoacoustic spectroscopy (Bukhov and Carpentier 2003) or 77 K excitation spectra of Chl fluorescence (Špunda *et al.* 1998a). This review is focused on the recent progress in understanding the mechanisms and photo-protective role of non-radiative dissipation of absorbed excitation energy and complements the former review concerning this topic that was published in *Photosynthetica* (Pospíšil 1997).

Non-photochemical quenching of Chl *a* fluorescence

Upon constant irradiation of a photosynthetic material in dark-adapted state, a typical time course of the yield of Chl fluorescence is observed. Such characteristic fluorescence time course has been termed the Kautsky effect (Kautsky and Hirsch 1931). Initially the fluorescence yield increases and reaches the maximum within tenths of second to seconds. Subsequently the fluorescence level starts to fall over a time-scale of a few minutes. This decrease is termed Chl fluorescence quenching. In this period the light-induced activation of enzymes involved in carbon metabolism occurs and consequently the photosynthetic electron transport activity is increased. Excitation energy is utilized in photochemical reactions, *i.e.* the charge separation in PS2 RCs followed by an electron transport *via* a set of carriers. Thus, a corresponding decrease of the fluorescence yield is referred to as photochemical quenching. All other processes that also cause a decrease of Chl fluorescence are collectively called non-photochemical quenching (NPQ). Photochemical and non-photochemical quenching can be differentiated by means of saturation pulse method (Schreiber *et al.* 1986). For quantification of the quenching processes various fluorescence parameters can be used (for reviews, see Maxwell and Johnson 2000, Roháček 2002).

Different processes that contribute to NPQ relax at different rates in darkness following a period of irradiation. The kinetics of relaxation of these different processes can be therefore used to distinguish them (Demmig

and Winter 1988, Horton and Hague 1988, Quick and Stitt 1989, Walters and Horton 1991). The relaxation of NPQ was monitored using periodic saturating pulses of photons in barley leaves (Quick and Stitt 1989, Walters and Horton 1991). Three components of NPQ were found with half times of about 1 min, 5 min, and hours (Quick and Stitt 1989) and 1 min, 5–10 min, and more than 30 min (Walters and Horton 1991). Using the same technique, similar results were obtained with isolated barley protoplasts (Horton and Hague 1988). Three components with half times of 30 s, 8 min, and about 30 min were observed.

The fast component is called energy-dependent quenching (q_E) due to its dependence on the energization of thylakoid membrane (Horton *et al.* 1996, Müller *et al.* 2001). q_E is a process of heat dissipation (or non-radiative dissipation) which is important in the protection of the photosynthetic apparatus against photo-oxidative damage under excess irradiation. For induction of maximal q_E , both low thylakoid lumen pH and the presence of xanthophylls are necessary.

The middle component, q_T , is associated with the phenomenon of state transitions (Allen and Forsberg 2001, Haldrup *et al.* 2001, Wollman 2001) and is therefore called state-transition quenching. State transitions are short-term processes that change the antenna sizes of PS2 and PS1. q_T induction and reversal caused by these alterations occur over time-scales ranging from minutes to tens

of minutes (McCormac *et al.* 1994). The basis of state transitions lies in the reversible phosphorylation of light-harvesting complex of PS2 (LHC2) and the movement of phosphorylated LHC2 from PS2 to PS1. The decrease of the PS2 antenna size is accompanied by a reduction of the amount of excitation energy in PS2 with a corresponding decrease in the PS2 fluorescence intensity. Unlike q_E , an involvement of q_T in photo-protection is unclear. It was suggested that LHC2 phosphorylation protects the photosynthetic apparatus against photoinhibition (Horton and Lee 1985). However, some studies show that LHC2 phosphorylation is inhibited upon short-term exposure to high irradiance (HI). This effect was observed in higher plants both *in vivo* (Demmig *et al.* 1987, Rintamäki *et al.* 1997, Pursiheimo *et al.* 2001) and in intact chloroplasts (Ebbert and Godde 1994). A contribution of q_T to the overall NPQ is rather negligible under HI stress in most plants

(Müller *et al.* 2001). q_T seems to be important only in low irradiances, when it regulates the distribution of excitation energy between PS1 and PS2 and thereby optimizes the photosynthetic reactions. As q_T does not represent dissipation of excitation energy *via* heat, we will not deal with this component of Chl fluorescence quenching in more detail.

Whereas the fast component of NPQ saturates at HI, the slow component increases as the irradiance is increased (Walters and Horton 1991). The slow component is caused by photoinhibition and is therefore called photoinhibitory quenching denoted as q_I (Krause 1988, Ruban and Horton 1995). This quenching shows the relaxation in the range of hours to days and reflects both slowly relaxing photo-protective processes and damage to PS2 RCs under stress conditions.

Energy-dependent quenching (q_E)

Role of thylakoid lumen pH and xanthophylls in q_E :

When the absorption of photon energy exceeds the capacity of the dark reactions to utilize ATP and NADPH produced by a photosynthetic electron transport, syntheses of ATP and NADPH are limited. This subsequently leads to a decrease in pH within the thylakoid lumen and the feedback regulation of light-harvesting by triggering the dissipation of excess absorbed energy as heat. The control by lumen pH allows induction and reversal of the energy dissipation within seconds to minutes. Low lumen pH does not have to be generated by light-dependent reactions. Using isolated thylakoids it is possible to induce q_E in darkness by simply lowering the pH of the buffer or by generating a pH gradient across the thylakoid membrane (ΔpH) by ATP hydrolysis-dependent proton pumping (Gilmore and Yamamoto 1992). Thus, q_E depends directly on lumen pH and the role of photon energy in q_E induction is indirect.

q_E is associated with the inter-conversion of xanthophyll pigments in the so-called xanthophyll cycle (Fig. 1, for reviews, see Pfündel and Bilger 1994, Demmig-Adams and Adams 1996a, Eskling *et al.* 1997, Gilmore 1997, Morosinotto *et al.* 2003). Low lumen pH activates the enzyme violaxanthin (V) de-epoxidase which converts V to zeaxanthin (Z) *via* the intermediate antheraxanthin (A) (Pfündel and Bilger 1994, Eskling *et al.* 1997). V de-epoxidation can be blocked by an inhibitor of V de-epoxidase, dithiothreitol (Yamamoto and Kamite 1972). Blocking this de-epoxidation reaction by dithiothreitol results in inhibition of q_E (for example, see Bilger and Björkman 1990, Demmig-Adams *et al.* 1990). In addition to the inhibitor studies, the requirement for the de-epoxidized xanthophylls in q_E has been proved using xanthophyll cycle mutants. The *npq1* mutants of the unicellular green alga *Chlamydomonas reinhardtii* and the C_3 vascular plant *Arabidopsis thaliana* that are unable to convert V to A and Z have lower levels of q_E compared to

the wild type (Niyogi *et al.* 1997a, 1998). The involvement of Z and A formation *via* the xanthophyll cycle in q_E was also confirmed in studies using V de-epoxidase antisense tobacco plants which exhibit suppressed ability to form Z and A (Chang *et al.* 2000, Sun *et al.* 2001, Verhoeven *et al.* 2001). As *npq1* mutants, these transgenic plants have reduced q_E compared to the wild-type plants.

q_E correlates better with the amount of Z and A than with the amount of Z alone (Gilmore and Yamamoto 1993, Demmig-Adams and Adams 1996b, Demmig-Adams *et al.* 1996a, Niyogi *et al.* 1997a). These findings support the idea that both A and Z are involved in q_E . Furthermore, the marine prasinophycean alga *Mantoniella squamata* has an incomplete xanthophyll cycle, when under HI *in vivo* V is converted to A without the formation of Z. However, this primitive green alga still exhibits uncoupler- and dithiothreitol-sensitive NPQ, *i.e.* the features of q_E in higher plants (Goss *et al.* 1998, Gilmore and Yamamoto 2001).

In addition to A and Z, also a third xanthophyll, lutein (L), has been implicated in q_E . The *Chlamydomonas lor1* mutant, which lacks L and luteoxanthin, shows lower q_E than the wild type (Niyogi *et al.* 1997b). Similarly, the *Arabidopsis lut2* mutant lacks L and exhibits lower q_E (Pogson *et al.* 1998, Lokstein *et al.* 2002). Almost complete inhibition of q_E was observed for the *Chlamydomonas npq1 lor1* and *Arabidopsis npq1 lut2* double mutants that are unable to convert V to A and Z and are also defective in the synthesis of L (Niyogi *et al.* 1997b, 2001). Furthermore, by modifying the biosynthesis of L, Pogson and Rissler (2000) found that increase in L content caused a slight, but significant increase in the rate of q_E induction despite a reduction in the xanthophyll cycle pool size in comparison with the wild-type plants. The mechanism by which L contributes to NPQ is still a matter of discussion (Niyogi *et al.* 1997b, 2001, Pogson *et al.*

1998, Lokstein *et al.* 2002). L may have a direct role in the quenching of excited singlet Chl *a*. This is energetically feasible, since L has the energy level of the lowest singlet excited state below that of Chl *a* (Josue and Frank 2002, Polívka *et al.* 2002; see Table 1). Alternatively, the absence of L in *lut2* and *lor1* mutants could affect q_E indirectly through a perturbation of LHC2 structure. Indeed, L-deficient mutants are characterized by reduced stability of trimeric LHC2 and smaller PS2 antenna size relative to the wild-type plants (Niyogi *et al.* 2001, Lokstein *et al.* 2002).

The xanthophylls are necessary for the induction of maximal q_E , but not sufficient. In *npq2* mutants of *Chlamydomonas* and *Arabidopsis* that accumulate Z consti-

tutively, q_E must still be induced by low lumen pH (Niyogi 1999). This implies that the low lumen pH has an additional role in q_E , besides the activation of V de-epoxidase leading to the formation of A and Z. A conformational change in the thylakoid membrane may be involved in q_E (Horton *et al.* 1996). Lowering the lumen pH is necessary for this conformational change, together with V de-epoxidation. The acidification of the lumen results in the protonation of PS2 antenna pigment-protein complexes. Binding of protons and de-epoxidized xanthophylls to the LHC2 proteins causes the conformational change that can be monitored by an absorbance change at 535 nm (Ruban *et al.* 1993, Bilger and Björkman 1994). The origin of this absorbance change was

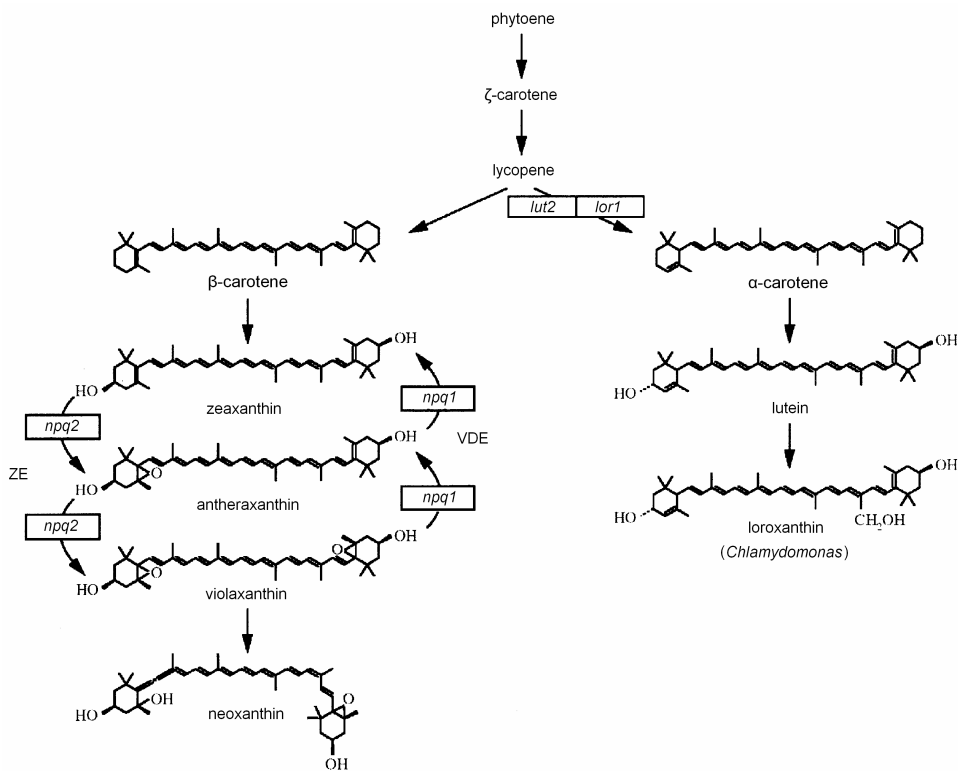


Fig. 1. The pathway for carotenoid biosynthesis in green algae and plants. The xanthophyll cycle operates in the β -carotene branch of the pathway and involves the di-epoxide violaxanthin, the mono-epoxide antheraxanthin, and the epoxide-free zeaxanthin. De-epoxidation is triggered under excess irradiance and is catalyzed by the enzyme violaxanthin de-epoxidase (VDE). The reverse reaction, epoxidation, occurring under non-excessive irradiance is catalyzed by zeaxanthin epoxidase (ZE). The reactions that are blocked in the *npq1*, *npq2*, *lut2*, and *lor1* mutants are also indicated (according to Baroli and Niyogi 2000).

Table 1. Electronic energies of the lowest singlet excited (S_1) states of xanthophylls. $S_0 \rightarrow S_1$ (Q_y) transition energy of Chl *a*: 14880 cm^{-1} (Josue and Frank 2002).

S_1 state energies [cm^{-1}]			Method	Reference
violaxanthin	zeaxanthin	lutein		
14470 \pm 90	14030 \pm 90		absorption spectroscopy, xanthophylls in solution	Polívka <i>et al.</i> 1999
14880 \pm 90	14550 \pm 90		fluorescence spectroscopy at room temp., xanthophylls in solution	Frank <i>et al.</i> 2000
15580 \pm 60	14610 \pm 40	14570 \pm 70	fluorescence spectroscopy at 77 K, xanthophylls in solution	Josue and Frank 2002
13700 \pm 300	13850 \pm 200	14050 \pm 300	absorption spectroscopy, xanthophylls in recombinant monomeric LHC2	Polívka <i>et al.</i> 2002

elucidated by Ruban *et al.* (2002). Using resonance Raman spectroscopy, they revealed that this absorbance change is caused by a large red shift in the absorption spectrum of one to two molecules of Z. Conformational changes have also been inferred from measurements of Chl *a* fluorescence lifetime distributions, which depend on the molecular environment of the excited Chl *a* (Gilmore 1997). The non-protonated state is characterized by a fluorescence lifetime component of 2 ns. The presence of ΔpH alone causes a fluorescence lifetime shift from 2.0 to 1.6 ns. This shift likely reflects a protonation-induced conformational change that is independent of xanthophyll cycle activity. Binding of Z and A to a protonated protein reduces the fluorescence lifetime to 0.4 ns. Thus, the amplitude of the 0.4 ns component is proportional to the concentration of Z and A.

Involvement of individual PS2 proteins in q_E : Formerly the minor LHC2 proteins CP26 (Lhcb5) and CP29 (Lhcb4) were suggested to be involved in q_E based on the following facts. First, Bassi *et al.* (1993) reported that the majority of the xanthophyll cycle pigments (approximately 80 %) are concentrated in these minor proteins. Second, CP26 and CP29 proteins bind *N,N'*-dicyclohexylcarbodiimide (DCCD) (Walters *et al.* 1994), an inhibitor of q_E which binds to carboxy groups that are involved in proton binding. DCCD-binding sites were identified in both CP26 (Walters *et al.* 1996) and CP29 (Pesaresi *et al.* 1997). Depletion of CP26 and, to some extent, CP29 was suggested to explain the q_E defect of tobacco mutants that are deficient in the PS2 core protein PsbZ, which is necessary for maintaining the stability of PS2-LHC2 super-complexes (Swiatek *et al.* 2001). The PsbZ-deficient plants show a greatly reduced capacity for NPQ under adverse growth conditions such as increased irradiance and/or decreased temperature. Under these growth conditions the extent of NPQ decreased in the PsbZ-deficient plants, in contrast to the wild type, when the acclimation to conditions increasing PS2 excitation pressure induces a typical response, namely increase in the extent of NPQ. However, Andersson *et al.* (2001) demonstrated that neither CP26 nor CP29 are the crucial sites for q_E using antisense *A. thaliana* plants lacking CP26 or CP29 proteins. Both CP26 and CP29 antisense plants revealed just slightly reduced capacity of q_E and moderately slowed-down formation and relaxation of NPQ in comparison with the wild-type plant. Moreover, V de-epoxidation was not affected in these antisense plants.

To identify proteins involved in q_E , Li *et al.* (2000) isolated mutants of *A. thaliana* that were defective in q_E , but had normal xanthophyll contents. Such *Arabidopsis npq4* mutants were characterized by the same extent and kinetics of Z accumulation upon HI treatment compared to the wild type (Li *et al.* 2000, 2002a, Graßes *et al.* 2002). The kinetics and extent of NPQ in the *npq4* mutant is similar to those of the *npq1 lut2* double mutant

lacking both Z and L (Niyogi *et al.* 2001). Li *et al.* (2000) revealed that the *npq4* mutant is defective in the nuclear gene encoding PsbS, a 22 kDa PS2 subunit (also called CP22). In addition to the absence of q_E , the *npq4* mutant also lacks the ΔpH - and xanthophyll cycle-dependent conformational change in the thylakoid membrane, monitored by an absorbance change at 535 nm that is necessary for q_E (Li *et al.* 2000). This indicates that binding of protons and/or Z to the PsbS protein may be a necessary feature of the q_E mechanism. The candidate proton-binding sites on the lumen side of PsbS involved in q_E have been identified (Li *et al.* 2002c). In addition, binding of Z to PsbS is responsible for the q_E -related 535 nm absorption change (Aspinall-O'Dea *et al.* 2002). The depletion of PsbS has no effect on photosynthetic performance and the contents of the other LHC2 proteins (Li *et al.* 2000, Graßes *et al.* 2002). The maximal PS2 photochemical efficiency (F_V/F_M) as well as 77 K emission spectra of Chl fluorescence in the *npq4* mutant are identical to those of the wild-type plant (Li *et al.* 2000). This implies that PsbS functions specifically in q_E and has not a structural role in the organization of the PS2 antenna.

The amount of the PsbS protein is a determinant of q_E capacity. Li *et al.* (2002a) compared homozygous wild-type *Arabidopsis* plants containing two *psbS* gene copies, heterozygous plants containing one *psbS* gene copy and homozygous *npq4* mutant plants containing no *psbS* gene copies. They found that heterozygous plants have 56 % of the wild-type *psbS* mRNA level and 58 % of the wild-type PsbS protein level. Importantly, this lower PsbS protein level results in a corresponding decrease in q_E to 60 % of the wild-type level. Thus, the extent of q_E is related to the amount of the PsbS protein, in addition to the concentration of de-epoxidized xanthophylls. Consistent with these findings, Ottander *et al.* (1995) observed increased amounts of the PsbS protein in thylakoids isolated from needles of Scots pine during winter. Similarly, the amount of PsbS increases during cold acclimation of Lodgepole pine grown under controlled conditions, and this increase was also associated with increased q_E (Savitch *et al.* 2002). It seems that increase in PsbS synthesis might be involved in photo-protection, when plants are growing at excess HI. The role of PsbS in formation of a quenching complex is, however, still unresolved. Taking into consideration the fact that PsbS appears to be unable to bind Chl (Dominici *et al.* 2002), a putative function of PsbS is the transduction of low lumen pH signal into a conformational change of neighbour LHC proteins where the quenching process could occur (Bassi and Caffarri 2000, Dominici *et al.* 2002).

Elrad *et al.* (2002) characterized a mutant of the green alga *Ch. reinhardtii*, designated *npq5*, which has a phenotype similar to that of the *Arabidopsis npq4* mutant. The *Chlamydomonas npq5* mutant is defective in its ability to establish q_E and shows the same kinetics and extent of V de-epoxidation during exposure to HI as the wild-type

cells. The *npq5* mutant lacks a major light-harvesting polypeptide of PS2 (Lhcbm1) suggesting that the major peripheral antenna, LHC2b, is required for the cells to elicit q_E . Thus, there may be differences between organisms in the specific antenna components that are involved in q_E . It is still unclear whether *Chlamydomonas* contains the PsbS protein necessary for q_E development in higher plants. None of the known genes encodes a polypeptide with high sequence similarity to PsbS (Elrad *et al.* 2002).

The major light-harvesting complex of PS2 (LHC2b) was also proposed to be involved in q_E mechanism in higher plants. Chow *et al.* (2000) observed in intermittent-irradiance-grown plants transferred to continuous irradiation a correlation between increasing contents of Chl *b* and LHC2b and NPQ, suggesting a role for LHC2b in thermal dissipation of absorbed photon energy. Another support comes from the observations that the antisense *Arabidopsis* plants lacking the Lhcb1 and Lhcb2 proteins possess reduced capacity for q_E compared to the wild type, in spite of the unchanged xanthophyll cycle activity (Andersson *et al.* 2003). However, it remains to be determined whether this reduction is due to a decrease in the number of quenching sites or a decrease in connectivity between LHC2b and the dissipation site. Alternatively, as these antisense plants have a reduced level of PsbS, the decreased q_E capacity may be solely a result of the lower PsbS amount.

Photophysical mechanism of q_E : Identification of a quencher of excitation energy is still a matter of debate. Nowadays there is a general agreement that conformational change within LHC2 is involved in q_E mechanism. One proposal is that this conformational change allows for a direct downhill energy transfer from the singlet excited state of Chl ($^1\text{Chl}^*$) to Z, followed by a rapid harmless dissipation of this excitation energy in the form of heat, when the excited singlet Z returns to the ground state by internal conversion (Polívka *et al.* 2002, Morosinotto *et al.* 2003). This proposal was previously based on the fact that conversion of V to Z is accompanied by the lowering of excited state energy level (Frank *et al.* 1994), thereby forming an energetically favourable pathway for the $^1\text{Chl}^*$ de-excitation.

The energy levels of the lowest singlet excited (S_1) state of V and Z were determined directly using femto-second absorption spectroscopy of the $S_1 \rightarrow S_2$ transitions (Polívka *et al.* 1999, 2002) and fluorescence spectroscopy of the $S_1 \rightarrow S_0$ transitions (Frank *et al.* 2000, Josue and Frank 2002) (Table 1). According to these studies (except Josue and Frank 2002), both V and Z could potentially accept energy from $^1\text{Chl}^*$ and, moreover, the energy difference between the S_1 states of V and Z may be too small to account for their different quenching capabilities. The best estimates of S_1 state energies of xanthophylls *in vivo* were performed by Polívka *et al.* (2002), since they determined S_1 energies in recombinant LHC2, in contrast to the other studies, in which S_1 energies were determined

for pigments in organic solvents. The S_1 energies of V and Z determined in their LHC2 protein environment are essentially the same (Polívka *et al.* 2002). Other factors such as distance and orientation of the xanthophylls relative to Chl are therefore more important in determining if direct singlet-singlet energy transfer occurs than energetic differences between these xanthophylls. Thus, xanthophyll cycle does not operate to lower the S_1 state energy level of xanthophylls but rather to bring about the conformational change of LHC2 proteins evoked by the binding of Z. Direct quenching of $^1\text{Chl}^*$ by Z has been recently supported by Ma *et al.* (2003), who additionally suggested other possible mechanism for Z involvement in $^1\text{Chl}^*$ quenching that lies in the formation of a heterodimer between Chl and Z.

A second hypothesis attributes to the xanthophylls an indirect role in q_E . Z mediates a structural change of LHC2 proteins, which may facilitate the $^1\text{Chl}^*$ de-excitation by internal conversion of Chl itself to the ground state, releasing excitation energy as heat (Horton *et al.* 1996). This process could involve formation of Chl-Chl dimers allowing thermal de-excitation (Crofts and Yerkes 1994) and require the formation of LHC2 aggregates (Horton *et al.* 1991). The aggregation of LHC2 increases interactions between Chl molecules belonging to neighbouring proteins and enhances the amplitude of q_E (Bassi and Caffarri 2000). The amplifying effect of LHC2 aggregation on q_E amplitude has been supported by Moya *et al.* (2001) who reported that LHC2 proteins incorporated into liposomes showed a greater quenching of Chl fluorescence than the same proteins in detergent solutions. Moreover, they demonstrated that increase of the protein density in the liposomes yielded a further fluorescence quenching to the extent typical for leaves.

Photo-protective function of q_E : q_E is important for photo-protection by $^1\text{Chl}^*$ quenching, which results in harmless dissipation of excess excitation energy as heat. Up to 80 % of the Chl excited states can be dissipated into heat, thereby protecting PS2 RCs from over-excitation (Bassi and Caffarri 2000). Intersystem crossing is one of possible pathways of $^1\text{Chl}^*$ de-excitation, by which the excited triplet Chl ($^3\text{Chl}^*$) is formed. $^3\text{Chl}^*$ can interact with ground-state triplet oxygen to produce singlet excited oxygen ($^1\text{O}_2^*$), an extremely damaging reactive oxygen species (Fig. 2; Baroli and Niyogi 2000, Demmig-Adams and Adams 2000, Niyogi 2000). Thus, q_E decreases the lifetime of $^1\text{Chl}^*$ and consequently the probability of $^1\text{O}_2^*$ generation in LHC2. Further, by decreasing the efficiency of energy transfer to PS2 RCs, q_E may also prevent the over-reduction of the electron transport chain resulting in the recombination of the primary radical pair, $\text{P680}^+\text{Pheo}^-$, which can generate the excited triplet states of both the accessory Chl molecule located in the D1 protein and P680, the latter as a minor population (Aro *et al.* 1993, Noguchi 2002). As in LHC2, interaction between $^3\text{Chl}^*$ and O_2 within PS2 RCs results

in formation of $^1\text{O}_2^*$. At last, q_E may also prevent the over-acidification of the thylakoid lumen that can inhibit the electron transport at PS2 donor side resulting in the generation of long-lived P680^+ and/or Y_Z^+ (oxidized secondary electron donor of PS2) (Niyogi 1999). P680^+ and Y_Z^+ are themselves capable of oxidizing nearby pigments and proteins, causing the damage to PS2 RCs (Aro *et al.* 1993).

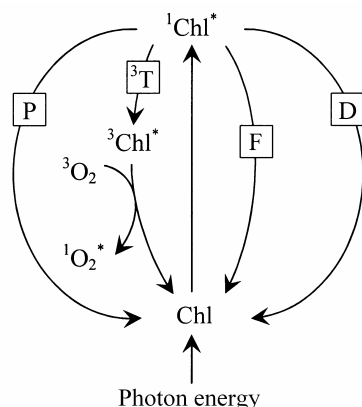


Fig. 2. Possible pathways of de-excitation of excited singlet chlorophyll (Chl) *a* molecules in photosystem 2 (PS2). Photon absorption results in conversion of Chl *a* from its ground state (Chl) to its excited singlet state ($^1\text{Chl}^*$). Excitation energy can be re-emitted as Chl *a* fluorescence (F), it can be transferred to reaction centres to drive photosynthetic electron transport (P), it can be de-excited by thermal dissipation process (D), or it can decay *via* the triplet pathway (^3T). $^1\text{Chl}^*$ can be converted to excited triplet Chl ($^3\text{Chl}^*$) through intersystem crossing. $^3\text{Chl}^*$ can transfer energy to ground-state triplet oxygen ($^3\text{O}_2$) to produce singlet oxygen ($^1\text{O}_2^*$) causing photo-oxidative damage of PS2 (according to Demmig-Adams and Adams 2000).

q_E is rapidly inducible and hence important under variable irradiances where it quickly adjusts photon harvesting. In particular, it is critical upon sudden exposure to excess photons which is characteristic for plants growing in the forest understory, where they are exposed to short intense flashes called “sunflecks” (Adams *et al.* 1999). Li *et al.* (2002b) showed that in a short-term HI treatment (time-scale of hours), q_E capacity rather than xanthophyll composition is critical for photo-protection. They observed the same extent of photoinhibition in three q_E -deficient *Arabidopsis* mutants that differ in xanthophyll composition, *i.e.* *npq4* mutant with normal xanthophyll levels, *npq4 npq1* double mutant lacking Z, and *npq4 npq1 lut2* triple mutant that additionally lacks L. On the contrary, transgenic *Arabidopsis* plants with higher q_E capacity generated by over-expression of PsbS were more resistant to photoinhibition than the wild-type plants despite the same xanthophyll cycle pool size (Li *et al.* 2002b).

In addition to short-term response, q_E responds also to the long-term requirements for PS2 down-regulation. In comparison with low-irradiance acclimated plants, plants

acclimated to HI exhibit higher capacity of q_E (Park *et al.* 1996, Brugnoli *et al.* 1998, Demmig-Adams 1998, Špunda *et al.* 1998a). Increased capacity of q_E is associated with a larger pool of xanthophyll cycle pigments and enhanced capacity to de-epoxidize xanthophyll cycle pigments (Brugnoli *et al.* 1998, Demmig-Adams 1998). However, q_E , V convertibility, and the xanthophyll cycle pool size must not necessarily correlate across various plant species. In a comparative study focused on different irradiance acclimation strategies for barley and Norway spruce plants, a greater extent of q_E for Norway spruce was found despite a significantly lower VAZ pool as compared to barley (Kurasová *et al.* 2003).

There are several indications that among the plants of North Temperate Zone the photosynthetic apparatus of evergreen conifers responds to the excess photons particularly by the elevated capacity of q_E (Huner *et al.* 1993, Ottander *et al.* 1995, Špunda *et al.* 1998a, Savitch *et al.* 2002, Kurasová *et al.* 2003). Other specific feature of Norway spruce plants is that HI acclimated plants exhibit significantly greater efficiency of V de-epoxidation (more than 90 %) than is usual for other plant species with corresponding VAZ pool size and LHC2 size (Kurasová *et al.* 2003). Thus, as indicated also by different shape of 77 K emission spectrum of Chl *a* fluorescence (Kalina *et al.* 1997, Špunda *et al.* 1998b), it seems that different organization of PS2 antenna as compared to other plant species allows efficient irradiation-induced q_E and facilitates V de-epoxidation in Norway spruce needles. On the contrary, it is reasonable that plants responding to HI mainly by increased photochemical de-excitation together with elevated photosynthetic activity develop PS2 organization that favours the excitation energy transfer to the PS2 core but reduces the capacity of q_E . Thus although q_E represents a photo-protective mechanism for all higher plants, its significance strongly depends on the eco-physiological strategy of the individual plant species.

In addition, q_E maintains the optimal rates of photon energy utilization and consequently affects overall photosynthetic productivity. Graßes *et al.* (2002) report that the PsbS protein-less mutants of *A. thaliana* exhibit a reduction in the growth rate when compared to wild type. Further, Külheim *et al.* (2002) observed that q_E affects net plant fitness. *Arabidopsis npq1* and *npq4* mutants grown under field conditions and fluctuating irradiation in a controlled environment produced less fruits and seeds per plant and also less seeds per fruit.

q_E is one member of a photo-protective system in higher plants and contributes to photo-protection to a various degree in different plant species. Although q_E may be an important mechanism for the resistance of the photosynthetic apparatus against HI stress, the following examples illustrate that photo-protection is not strictly dependent on q_E . Sun *et al.* (2001) reported that V de-epoxidase antisense tobacco plants did not exhibit greater susceptibility to photoinhibition than wild type under field conditions. Havaux *et al.* (2000) found that

developing leaves of *Arabidopsis npq1* mutant were tolerant to HI stress and $^1\text{O}_2^*$ toxicity induced by the photosensitizing dye eosin, a known generator of $^1\text{O}_2^*$. Further, the rates of HI-induced degradation of the D1 protein were the same for both *Arabidopsis npq4* mutant and wild-type plant (Graßes *et al.* 2002). Other photo-protective mechanisms therefore can efficiently compensate for the lack of q_E , such as antioxidant molecules including carotenoids, ascorbate, glutathione, and α -tocopherol, and antioxidant enzymes including superoxide dismutase, ascorbate peroxidase, glutathione reductase, and monodehydroascorbate reductase (Niyogi 1999). An enhanced capacity to scavenge reactive oxygen species has been observed during acclimation to HI (Grace and Logan 1996, Logan *et al.* 1996, 1998a,b, García-Plazaola and Becerril 2000, Hansen *et al.* 2002). In addition to enhanced capacity of q_E , increases in contents and activities of antioxidants represent another important long-term acclimation mechanism which helps to minimize damage to photosynthetic apparatus that could be caused by the absorption of excess photons.

The conversion of V to A and Z is also involved in other photo-protective mechanisms. Havaux and Niyogi (1999) and Havaux *et al.* (2000) showed that the xanthophyll cycle specifically protects thylakoid membranes

against lipid peroxidation. Upon photoinhibitory treatment, the *Arabidopsis npq1* mutant, that is unable to convert V to A and Z, exhibited more pronounced lipid peroxidation than the wild-type plant. Conversely, the *Arabidopsis npq4* mutant that is defective in q_E but possesses a normal xanthophyll cycle activity was tolerant to lipid peroxidation. These results demonstrated that photo-protection mediated by the xanthophyll cycle is not solely a result of the involvement of Z in q_E . Consistent with these observations, Davison *et al.* (2002) found that *Arabidopsis* plants over-expressing β -carotene hydroxylase, which catalyses the conversion of β -carotene to Z, showed reduced lipid peroxidation compared to the wild type under stress conditions. Thus, Z is important in the termination of lipid peroxidation chain reactions and decreases the thylakoid membrane fluidity (Tardy and Havaux 1997). The decrease in fluidity could be important by lowering the penetration of reactive oxygen species inside the thylakoid. In addition, Z may directly scavenge reactive oxygen species (Baroli *et al.* 2003). Antioxidant roles of Z become evident only during longer-term HI stress (time-scale of days) (Li *et al.* 2002b). And Z participates in the protection of photo-damaged and disassembled PS2 core complexes during PS2 repair cycle (Jin *et al.* 2001, 2003).

Photoinhibitory quenching (q_I): mechanisms and photo-protection

Under HI, when the protective capacity of q_E is saturated, a slowly-relaxing component of NPQ, termed q_I , becomes significant. In contrast to q_E , q_I is much less characterized. This form of non-photochemical fluorescence quenching is associated with photoinhibition and it comprises both the processes that contribute to protection of the photosynthetic apparatus against the effects of excess photons and also contributions from PS2 photo-damage. As given below, q_I is extremely heterogeneous.

The photo-protective component of q_I , like q_E , reduces HI induced damage in PS2 by dissipating excess absorbed photon energy and it appears to possess some characteristics of q_E . Some part of q_I may be associated with ΔpH , which is necessary for q_E induction. Gilmore and Yamamoto (1992) have suggested that ΔpH might be maintained in darkness by the reverse pumping of protons from the stroma to the lumen catalyzed by the ATP synthase. However, this is probably only the case when q_I is induced by low temperature stress (Ruban and Horton 1995). At higher temperatures, Ruban and Horton (1995) observed that a part of q_I is reversed by application of the uncoupler nigericin. But this effect was not related to a persistence of ΔpH , as was shown by the measurement of 9-aminoacridine fluorescence. Instead, they have suggested that the nigericin-sensitive component of q_I is due to stable protonation of LHC2.

For efficient q_E , the inter-conversion of xanthophylls in the xanthophyll cycle together with the formation of ΔpH is necessary. The involvement of the xanthophyll

cycle activity in q_I was also reported such that relaxation of q_I has been correlated with the conversion of Z and A back to V (Jahns and Mieke 1996, Verhoeven *et al.* 1996, Demmig-Adams *et al.* 1998). Färber *et al.* (1997) studied the function of Z in q_I by measuring the xanthophyll cycle activity in different antenna sub-complexes of PS2. They proposed that Z binding to CP29 protein is related to the generation of quenching centres that are involved in q_I .

Sustained forms of xanthophyll cycle-dependent energy dissipation were observed in over-wintering plants transferred from subfreezing temperatures in the field prior to sunrise to room temperature (Verhoeven *et al.* 1998, 1999a). The form that relaxes rapidly upon warming (time-scale of hours) may result from a maintained ΔpH due to low temperature sustained activity of ATPase that catalyzes reverse proton transport into the thylakoid lumen (Gilmore 1997). Also low temperature-induced decrease of thylakoid membrane permeability affecting proton leakage from the lumen to the stroma may contribute to the maintenance of ΔpH (Verhoeven *et al.* 1998). In addition to this rapidly relaxing q_I , more slowly relaxing type of q_I does not depend on the ΔpH and requires days for complete relaxation. This type of q_I seems to be associated with a reorganization of LHC2 proteins into aggregates allowing a stable engagement of Z+A in thermal energy dissipation at low temperatures (Ottander *et al.* 1995, Verhoeven *et al.* 1999a). The involvement of such considerable structural changes of pigment-protein

complexes in q_I for cold-acclimated evergreens was supported by Gilmore and Ball (2000). They reported ΔpH independent mechanism for the sustained thermal energy dissipation during winter acclimation of snow gum seedlings consisting in formation of special energy-dissipating complexes. The plants were characterized by a new specific band in the 77 K Chl fluorescence emission spectra centred around 715 nm which disappeared during relaxation of q_I associated with the epoxidation of Z+A to V. Retention of Z+A in darkness was also observed during acclimation of Norway spruce to HI in controlled environment (Kurasová *et al.* 2003). Persistent Z+A permits a rapid and effective induction of thermal energy dissipation upon sudden exposure to excess photons.

Furthermore, relaxation of q_I is accompanied by de-phosphorylation of thylakoid proteins. Whereas both D1/D2 and LHC2 proteins (presumably Lhcb1 protein) exhibit dark-sustained phosphorylation subsequent to HI stress, only dark-sustained D1/D2 phosphorylation closely correlates with q_I (Ebbert *et al.* 2001). Similarly, dark-sustained D1/D2 phosphorylation was also observed in over-wintering plants that exhibit predawn retention of de-epoxidized xanthophylls (Z and A) and concomitant sustained lowering of PS2 efficiency (F_v/F_m) during the winter (Adams *et al.* 2001). The possibility that protein phosphorylation is involved in q_I has been supported by Xu *et al.* (1999) and Ebbert *et al.* (2001), who showed that phosphatase inhibitors causing the blockage of protein de-phosphorylation inhibit Z epoxidation and relaxation of q_I during recovery from photoinhibition.

In addition to antenna-localized q_I associated with xanthophyll cycle activity, a part of q_I may be located at the PS2 RCs (Richter *et al.* 1999, Bukhov *et al.* 2001). Richter *et al.* (1999) proposed that this type of quenching involves non-radiative decay of the primary charge separated state ($P680^+Pheo^-Q_A^-$) to its ground state ($P680PheoQ_A^-$) and/or triplet excited state [$^3(P680^+Pheo^-)Q_A^-$] in the photo-damaged PS2 RCs. A specific mechanism for thermal dissipation of excess excitation energy within PS2 RCs has been recently introduced for cold-acclimated plants (Ivanov *et al.* 2002, 2003, Sane *et al.* 2003).

The triplet Chl pathway

In addition to non-radiative S_1-S_0 Chl *a* de-excitation pathway, there is another non-photochemical mechanism for quenching of excited Chls. As shown in Fig. 2, the excited singlet Chl ($^1Chl^*$) can be converted into the excited triplet Chl ($^3Chl^*$), a long-lived state that is incapable of initiating photosynthetic electron transport. $^3Chl^*$ is efficiently quenched by triplet-triplet energy transfer to carotenoids, which rapidly releases excitation energy in the form of heat (Siefermann-Harms 1987, Demmig-Adams *et al.* 1996b, Baroli and Niyogi 2000). Peterman *et al.* (1997) estimated the efficiency of the triplet transfer from Chl *a* to xanthophylls in LHC2 monomers at 77 K to be $80 \pm 5\%$. Significantly higher efficiency of the triplet

Thermoluminescence measurements showed that the redox potentials of Q_A and Q_B were altered during cold acclimation. The characteristic maxima of Q ($S_2Q_A^-$ recombination) and B ($S_2Q_B^-$ recombination) bands were shifted to higher and lower temperatures, respectively. The shift in $S_2Q_A^-$ recombination to higher temperatures indicates increase of the free energy gap between $P680^+$ and Q_A^- corresponding to decrease of the probability for a charge recombination pathway involving the primary radical pair $P680^+Pheo^-$. Moreover, reduction of the difference between maxima of Q and B bands reflects a narrowing of the redox potential gap between Q_A and Q_B . This effect results in increased population of Q_A^- and thereby in increased probability of non-radiative charge recombination between Q_A^- and $P680^+$.

Aside from the clear photo-protective role of the xanthophyll-cycle dependent thermal energy dissipation within LHC2 complexes, thermal dissipation localized in PS2 RCs may have a photo-protective function and does not represent only photoinhibitory damage (Lee *et al.* 2001, Chow *et al.* 2002). Photoinhibition is characterized by the conversion of a fraction of PS2 RCs to a photochemically inactive state, in which they still act as efficient traps of excitation energy and convert this trapped energy to heat (Weis and Berry 1987, Krause 1988, Krieger *et al.* 1992). Öquist *et al.* (1992a,b) hypothesized that photo-inactivated PS2 complexes acting as sinks for excitation energy may help to prevent neighbouring PS2 complexes from photo-inactivation. This hypothesis was supported by Lee *et al.* (2001) who observed that about 20 % of PS2 RCs retained the photochemical activity after prolonged HI treatment despite the inhibition of D1 protein synthesis by lincomycin. This implied that this fraction was photo-protected by mechanisms other than PS2 repair cycle. They concluded that the residual population of functional PS2 was photo-protected by an abundance of photo-inactivated neighbours. Thus, in this model photon energy absorbed by active PS2 complexes is efficiently transferred to the photo-inactivated neighbours that act as strong sinks to dissipate the excitation energy as heat.

transfer at 77 K ($94 \pm 2\%$) was reported in trimeric LHC2 and, moreover, the transfer of triplet state energy from Chl to xanthophylls is essentially 100 % efficient at room temperature (Peterman *et al.* 1995).

4–25 % of the photons absorbed by PS2 can be dissipated *via* this triplet pathway (Foyer and Harbinson 1999). The proportion of photons dissipated in this way is correlated to the average lifetime of $^1Chl^*$. However, because the lifetime of $^1Chl^*$ is kept relatively low by q_E , dissipation *via* the triplet pathway is usually limited to the lower end of the estimated 4–25 % range (Niyogi 2000). The main difference between q_E and the triplet pathway is that q_E is inducible and subject to regulation, whereas the

triplet pathway is supposed to be constitutive and unregulated.

The involvement of the triplet Chl pathway in photo-protection is evident from Fig. 2, because the interaction of $^3\text{Chl}^*$ with the ground-state triplet oxygen ($^3\text{O}_2$) results in the generation of singlet oxygen ($^1\text{O}_2^*$), a highly reactive oxygen species. Thus, quenching of $^3\text{Chl}^*$ by carotenoids prevents the generation of $^1\text{O}_2^*$. Carotenoid triplets cannot react with $^3\text{O}_2$ to produce $^1\text{O}_2^*$, and they decay harmlessly by thermal emission. Carotenoids possess another feature that is important with respect to

photo-protection, namely they may also quench $^1\text{O}_2^*$ (Demmig-Adams *et al.* 1996b, Baroli and Niyogi 2000). Interaction with $^1\text{O}_2^*$ (as with $^3\text{Chl}^*$) leads to the formation of a carotenoid triplet, which decays to the ground state by thermal dissipation. Croce *et al.* (1999) showed that L, Z, and V (in decreasing order of effectiveness) are involved in protecting against Chl photo-bleaching by quenching both $^3\text{Chl}^*$ and $^1\text{O}_2^*$, whereas neoxanthin functions mainly in $^1\text{O}_2^*$ quenching. β -carotene scavenges $^1\text{O}_2^*$ but it is unable to quench the triplet excited state of P680 (Telfer 2002).

Thermal energy dissipation within PS1

At HI, when photo-protective thermal dissipation of excess absorbed photon energy within PS2 (q_E) is engaged, PS1 absorbs more photons compared to the electron flux from PS2. This excess photon energy absorbed by PS1 can be dissipated *via* cyclic electron transport around PS1. Furthermore, cyclic electron flow may be involved in maintaining the ΔpH that is necessary for down-regulation of PS2 activity by q_E (Heber and Walker 1992).

Thermal energy dissipation in PS1 is much less studied than that in PS2. Antenna-type quenching mechanisms similar to those occurring in PS2 were not observed in PS1. However, it was recognized that PS1, like PS2, is a shallow trap and therefore PS1 and its antenna are essentially equilibrated (Croce *et al.* 1996). Furthermore, conversion of V to A and Z upon HI treatment takes place also in PS1 (Thayer and Björkman 1992, Lee and Thornber 1995, Färber *et al.* 1997). These findings imply that thermal dissipation mechanism in the PS1 antenna similar to q_E could be an efficient regulatory mechanism for energy utilization in PS1 (Verhoeven *et al.* 1999b). Regulation of the efficiency of excitation energy transfer within LHCs of PS1 was proved by means of

excitation spectra of Chl *a* fluorescence at 77 K (Špunda *et al.* 1998a).

Nevertheless, the main site for thermal dissipation in PS1 may be RCs rather than LHCs. The role of non-photochemical quencher of excited states in PS1 is attributed to the oxidized primary electron donor of PS1, P700^+ . Energy dissipation in PS1 is automatically regulated by the supply of electrons from PS2. In the absence of electron donors, P700^+ is stable for hours or longer. P700^+ is as effective quencher of excited states from the PS1 antenna as P700 (Nuijs *et al.* 1986). However, in the P700^+ state, PS1 photochemistry is not possible and the excited state energy is dissipated as heat. Thus, energy absorbed by PS1 which is in excess of the photochemical turnover of PS2 is automatically dissipated as heat (Owens 1996).

Barth *et al.* (2001) suggested that increased rate of charge recombination reactions in the PS1 RCs represents a photo-protective mechanism of harmless energy dissipation. Further, they observed that under HI stress a high proportion of P700 accumulates in the oxidized state. Presumably, conversion of excitation energy to heat by P700^+ may efficiently contribute to photo-protection.

References

- Adams, W.W., III, Demmig-Adams, B., Logan, B.A., Barker, D.H., Osmond, C.B.: Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open *Eucalyptus* forest. – *Plant Cell Environ.* **22**: 125-136, 1999.
- Adams, W.W., III, Demmig-Adams, B., Rosenstiel, T.N., Ebbert, V.: Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during the winter. – *Photosynth. Res.* **67**: 51-62, 2001.
- Allen, J.F., Forsberg, J.: Molecular recognition in thylakoid structure and function. – *Trends Plant Sci.* **6**: 317-326, 2001.
- Andersson, B., Aro, E.-M.: Photodamage and D1 protein turnover in photosystem II. – In: Aro, E.-M., Andersson, B. (ed.): *Regulation of Photosynthesis*. Pp. 377-393. Kluwer Academic Publ., Dordrecht – Boston – London 2001.
- Andersson, J., Walters, R.G., Horton, P., Jansson, S.: Antisense inhibition of the photosynthetic antenna proteins CP29 and CP26: Implications for the mechanism of protective energy dissipation. – *Plant Cell* **13**: 1193-1204, 2001.
- Andersson, J., Wentworth, M., Walters, R.G., Howard, C.A., Ruban, A.V., Horton, P., Jansson, S.: Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II – effects on photosynthesis, grana stacking and fitness. – *Plant J.* **35**: 350-361, 2003.
- Aro, E.-M., Virgin, I., Andersson, B.: Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. – *Biochim. biophys. Acta* **1143**: 113-134, 1993.
- Asada, K.: The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. – *Annu. Rev. Plant Physiol. Plant mol. Biol.* **50**: 601-639, 1999.
- Asada, K.: The water-water cycle as alternative photon and electron sinks. – *Phil. Trans. roy. Soc. London B* **355**: 1419-1431, 2000.
- Aspinall-O'Dea, M., Wentworth, M., Pascal, A., Robert, B., Ruban, A., Horton, P.: *In vitro* reconstitution of the activated zeaxanthin state associated with energy dissipation in plants. – *Proc. nat. Acad. Sci. USA* **99**: 16331-16335, 2002.
- Baier, M., Dietz, K.-J.: Alkyl hydroperoxide reductases: the way out of the oxidative breakdown of lipids in chloroplasts.

- Trends Plant Sci. **4**: 166-168, 1999.
- Bailey, S., Walters, R.G., Jansson, S., Horton, P.: Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses. – *Planta* **213**: 794-801, 2001.
- Barnes, J.D., Cardoso-Vilhena, J.: Interactions between electromagnetic radiation and the plant cuticle. – In: Kerstiens, G. (ed.): *Plant Cuticles and Integrated Functional Approach*. Pp. 157-174. BIOS Scientific Publishers, Oxford 1996.
- Baroli, I., Do, A.D., Yamane, T., Niyogi, K.K.: Zeaxanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress. – *Plant Cell* **15**: 992-1008, 2003.
- Baroli, I., Niyogi, K.K.: Molecular genetics of xanthophyll-dependent photoprotection in green algae and plants. – *Phil. Trans. roy. Soc. London B* **355**: 1385-1394, 2000.
- Barth, C., Krause, G.H., Winter, K.: Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. – *Plant Cell Environ.* **24**: 163-176, 2001.
- Bassi, R., Caffarri, S.: Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls. – *Photosynth. Res.* **64**: 243-256, 2000.
- Bassi, R., Pineau, B., Dainese, P., Marquardt, J.: Carotenoid-binding proteins of photosystem II. – *Eur. J. Biochem.* **212**: 297-303, 1993.
- Bilger, W., Björkman, O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. – *Photosynth. Res.* **25**: 173-185, 1990.
- Bilger, W., Björkman, O.: Relationships among violaxanthin deepoxidation, thylakoid membrane conformation, and non-photochemical chlorophyll fluorescence quenching in leaves of cotton (*Gossypium hirsutum* L.). – *Planta* **193**: 238-246, 1994.
- Björkman, O., Demmig-Adams, B.: Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. – In: Schulze, E.-D., Caldwell, M.M. (ed.): *Ecophysiology of Photosynthesis*. Pp. 17-47. Springer-Verlag, Berlin 1994.
- Brugnoli, E., Scartazza, A., De Tullio, M.C., Monteverdi, M.C., Lauteri, M., Augusti, A.: Zeaxanthin and non-photochemical quenching in sun and shade leaves of C₃ and C₄ plants. – *Physiol. Plant.* **104**: 727-734, 1998.
- Bukhov, N.G., Carpentier, R.: Measurement of photochemical quenching of absorbed quanta in photosystem I of intact leaves using simultaneous measurements of absorbance changes at 830 nm and thermal dissipation. – *Planta* **216**: 630-638, 2003.
- Bukhov, N.G., Heber, U., Wiese, C., Shuvalov, V.A.: Energy dissipation in photosynthesis: Does the quenching of chlorophyll fluorescence originate from antenna complexes of photosystem II or from the reaction center? – *Planta* **212**: 749-758, 2001.
- Chang, S.-H., Bugos, R.C., Sun, W.-H., Yamamoto, H.Y.: Antisense suppression of violaxanthin de-epoxidase in tobacco does not affect plant performance in controlled growth conditions. – *Photosynth. Res.* **64**: 95-103, 2000.
- Choudhury, N.K., Behera, R.K.: Photoinhibition of photosynthesis: Role of carotenoids in photoprotection of chloroplast constituents. – *Photosynthetica* **39**: 481-488, 2001.
- Chow, W.S., Funk, C., Hope, A.B., Govindjee: Greening of intermittent-light-grown bean plants in continuous light: Thylakoid components in relation to photosynthetic performance and capacity for photoprotection. – *Indian J. Biochem. Biophys.* **37**: 395-404, 2000.
- Chow, W.S., Lee, H.-Y., Park, Y.-I., Park, Y.-M., Hong, Y.-N., Anderson, J.M.: The role of inactive photosystem-II-mediated quenching in a last-ditch community defence against high light stress *in vivo*. – *Phil. Trans. roy. Soc. London B* **357**: 1441-1450, 2002.
- Croce, R., Weiss, S., Bassi, R.: Carotenoid-binding sites of the major light-harvesting complex II of higher plants. – *J. biol. Chem.* **274**: 29613-29623, 1999.
- Croce, R., Zucchelli, G., Garlaschi, F.M., Bassi, R., Jennings, R.C.: Excited state equilibration in the photosystem I-light-harvesting I complex: P700 is almost isoenergetic with its antenna. – *Biochemistry* **35**: 8572-8579, 1996.
- Crofts, A.R., Yerkes, C.T.: A molecular mechanism for q_E-quenching. – *FEBS Lett.* **352**: 265-270, 1994.
- Davison, P.A., Hunter, C.N., Horton, P.: Overexpression of β -carotene hydroxylase enhances stress tolerance in *Arabidopsis*. – *Nature* **418**: 203-206, 2002.
- Demmig, B., Winter, K.: Characterisation of three components of non-photochemical fluorescence quenching and their response to photoinhibition. – *Aust. J. Plant Physiol.* **15**: 163-177, 1988.
- Demmig, B., Cleland, R.E., Björkman, O.: Photoinhibition, 77 K chlorophyll fluorescence quenching and phosphorylation of the light-harvesting chlorophyll-protein complex of photosystem II in soybean leaves. – *Planta* **172**: 378-385, 1987.
- Demmig-Adams, B.: Survey of thermal energy dissipation and pigment composition in sun and shade leaves. – *Plant Cell Physiol.* **39**: 474-482, 1998.
- Demmig-Adams, B., Adams, W.W., III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. – *Trends Plant Sci.* **1**: 21-26, 1996a.
- Demmig-Adams, B., Adams, W.W., III: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. – *Planta* **198**: 460-470, 1996b.
- Demmig-Adams, B., Adams, W.W., III: Harvesting sunlight safely. – *Nature* **403**: 371-374, 2000.
- Demmig-Adams, B., Adams, W.W., III, Barker, D.H., Logan, B.A., Bowling, D.R., Verhoeven, A.S.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – *Physiol. Plant.* **98**: 253-264, 1996a.
- Demmig-Adams, B., Adams, W.W., III, Heber, U., Neimanis, S., Winter, K., Krüger, A., Czygan, F.-C., Bilger, W., Björkman, O.: Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. – *Plant Physiol.* **92**: 293-301, 1990.
- Demmig-Adams, B., Gilmore, A.M., Adams, W.W., III: *In vivo* functions of carotenoids in higher plants. – *FASEB J.* **10**: 403-412, 1996b.
- Demmig-Adams, B., Moeller, D.L., Logan, B.A., Adams, W.W., III: Positive correlation between levels of retained zeaxanthin+antheraxanthin and degree of photoinhibition in shade leaves of *Schefflera arboricola* (Hayata) Merrill. – *Planta* **205**: 367-374, 1998.
- Dominici, P., Caffarri, S., Armenante, F., Ceoldo, S., Crimi, M., Bassi, R.: Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant. – *J. biol. Chem.* **277**: 22750-22758, 2002.

- Ebbert, V., Demmig-Adams, B., Adams, W.W., III, Mueh, K.E., Staehelin, L.A.: Correlation between persistent forms of zeaxanthin-dependent energy dissipation and thylakoid protein phosphorylation. – *Photosynth. Res.* **67**: 63-78, 2001.
- Ebbert, V., Godde, D.: Regulation of thylakoid protein phosphorylation in intact chloroplasts by the activity of kinases and phosphatases. – *Biochim. biophys. Acta* **1187**: 335-346, 1994.
- Elrad, D., Niyogi, K.K., Grossman, A.R.: A major light-harvesting polypeptide of photosystem II functions in thermal dissipation. – *Plant Cell* **14**: 1801-1816, 2002.
- Eskling, M., Arvidsson, P.-O., Åkerlund, H.-E.: The xanthophyll cycle, its regulation and components. – *Physiol. Plant.* **100**: 806-816, 1997.
- Färber, A., Young, A.J., Ruban, A.V., Horton, P., Jahns, P.: Dynamics of xanthophyll-cycle activity in different antenna sub-complexes in the photosynthetic membranes of higher plants. The relationship between zeaxanthin conversion and non-photochemical fluorescence quenching. – *Plant Physiol.* **115**: 1609-1618, 1997.
- Foyer, C.H., Harbinson, J.: Relationships between antioxidant metabolism and carotenoids in the regulation of photosynthesis. – In: Frank, H.A., Young, A.J., Britton, G., Cogdell, R.J. (ed.): *The Photochemistry of Carotenoids*. Pp. 305-325. Kluwer Academic Publ., Dordrecht 1999.
- Frank, H.A., Bautista, J.A., Josue, J.S., Young, A.J.: Mechanism of non-photochemical quenching in green plants: energies of the lowest excited singlet states of violaxanthin and zeaxanthin. – *Biochemistry* **39**: 2831-2837, 2000.
- Frank, H.A., Cua, A., Chynwat, V., Young, A., Gosztola, D., Wasielewski, M.R.: Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. – *Photosynth. Res.* **41**: 389-395, 1994.
- García-Plazaola, J.I., Becerril, J.M.: Photoprotection mechanisms in European beech (*Fagus sylvatica* L.) seedlings from diverse climatic origins. – *Trees* **14**: 339-343, 2000.
- Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. – *Physiol. Plant.* **99**: 197-209, 1997.
- Gilmore, A.M., Ball, M.C.: Protection and storage of chlorophyll in overwintering evergreens. – *Proc. nat. Acad. Sci. USA* **97**: 11098-11101, 2000.
- Gilmore, A.M., Yamamoto, H.Y.: Dark induction of zeaxanthin-dependent nonphotochemical fluorescence quenching mediated by ATP. – *Proc. nat. Acad. Sci. USA* **89**: 1899-1903, 1992.
- Gilmore, A.M., Yamamoto, H.Y.: Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. – *Photosynth. Res.* **35**: 67-78, 1993.
- Gilmore, A.M., Yamamoto, H.Y.: Time-resolution of the antheraxanthin- and Δ pH-dependent chlorophyll *a* fluorescence components associated with photosystem II energy dissipation in *Mantoniella squamata*. – *Photochem. Photobiol.* **74**: 291-302, 2001.
- Goss, R., Böhme, K., Wilhelm, C.: The xanthophyll cycle of *Mantoniella squamata* converts violaxanthin into antheraxanthin but not to zeaxanthin: consequences for the mechanism of enhanced non-photochemical energy dissipation. – *Planta* **205**: 613-621, 1998.
- Grace, S.C., Logan, B.A.: Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. – *Plant Physiol.* **112**: 1631-1640, 1996.
- Graßes, T., Pesaresi, P., Schiavon, F., Varotto, C., Salamini, F., Jahns, P., Leister, D.: The role of Δ pH-dependent dissipation of excitation energy in protecting photosystem II against light-induced damage in *Arabidopsis thaliana*. – *Plant Physiol. Biochem.* **40**: 41-49, 2002.
- Haldrup, A., Jensen, P.E., Lunde, C., Scheller, H.V.: Balance of power: a view of the mechanism of photosynthetic state transitions. – *Trends Plant Sci.* **6**: 301-305, 2001.
- Hansen, U., Fiedler, B., Rank, B.: Variation of pigment composition and antioxidant systems along the canopy light gradient in a mixed beech/oak forest: a comparative study on deciduous tree species differing in shade tolerance. – *Trees* **16**: 354-364, 2002.
- Havaux, M., Bonfils, J.-P., Lütz, C., Niyogi, K.K.: Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the *npq1 Arabidopsis* mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. – *Plant Physiol.* **124**: 273-284, 2000.
- Havaux, M., Niyogi, K.K.: The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. – *Proc. nat. Acad. Sci. USA* **96**: 8762-8767, 1999.
- Heber, U., Walker, D.: Concerning a dual function of coupled cyclic electron transport in leaves. – *Plant Physiol.* **100**: 1621-1626, 1992.
- Horton, P., Hague, A.: Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching. – *Biochim. biophys. Acta* **932**: 107-115, 1988.
- Horton, P., Lee, P.J.: Phosphorylation of chloroplast membrane proteins partially protects against photoinhibition. – *Planta* **165**: 37-42, 1985.
- Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G., Young, A.J.: Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. – *FEBS Lett.* **292**: 1-4, 1991.
- Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. – *Annu. Rev. Plant Physiol. Plant mol. Biol.* **47**: 655-684, 1996.
- Huner, N.P.A., Öquist, G., Hurry, V.M., Król, M., Falk, S., Griffith, M.: Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. – *Photosynth. Res.* **37**: 19-39, 1993.
- Ivanov, A.G., Sane, P.V., Hurry, V., Król, M., Svishnikov, D., Huner, N.P.A., Öquist, G.: Low-temperature modulation of the redox properties of the acceptor side of photosystem II: photoprotection through reaction centre quenching of excess energy. – *Physiol. Plant.* **119**: 376-383, 2003.
- Ivanov, A.G., Sane, P.V., Zeinalov, Y., Simidjiev, I., Huner, N.P.A., Öquist, G.: Seasonal responses of photosynthetic electron transport in Scots pine (*Pinus sylvestris* L.) studied by thermoluminescence. – *Planta* **215**: 457-465, 2002.
- Jahns, P., Miehle, B.: Kinetic correlation of recovery from photoinhibition and zeaxanthin epoxidation. – *Planta* **198**: 202-210, 1996.
- Jin, E.S., Polle, J.E.W., Melis, A.: Involvement of zeaxanthin and of the Cbr protein in the repair of photosystem II from photoinhibition in the green alga *Dunaliella salina*. – *Biochim. biophys. Acta* **1506**: 244-259, 2001.
- Jin, E.S., Yokthongwattana, K., Polle, J.E.W., Melis, A.: Role of the reversible xanthophyll cycle in the photosystem II damage and repair cycle in *Dunaliella salina*. – *Plant Physiol.* **132**: 352-364, 2003.

- Josue, J.S., Frank, H.A.: Direct determination of the S_1 excited-state energies of xanthophylls by low-temperature fluorescence spectroscopy. – *J. phys. Chem. A* **106**: 4815-4824, 2002.
- Kalina, J., Čajánek, M., Špunda, V., Marek, M.V.: Changes of the primary photosynthetic reactions of Norway spruce under elevated CO_2 . – In: Mohren, G.M.J., Kramer, K., Sabaté, S. (ed.): *Impacts of Global Change on Tree Physiology and Forest Ecosystems*. Pp. 59-66. Kluwer Academic Publ., Dordrecht – Boston – London 1997.
- Kautsky, H., Hirsch, A.: Neue Versuche zur Kohlensäure-assimilation. – *Naturwissenschaften* **19**: 964, 1931.
- Krause, G.H.: Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. – *Physiol. Plant.* **74**: 566-574, 1988.
- Krieger, A., Moya, I., Weis, E.: Energy-dependent quenching of chlorophyll *a* fluorescence: effect of pH on stationary fluorescence and picosecond-relaxation kinetics in thylakoid membranes and Photosystem II preparations. – *Biochim. biophys. Acta* **1102**: 167-176, 1992.
- Kühlheim, C., Ågren, J., Jansson, S.: Rapid regulation of light harvesting and plant fitness in the field. – *Science* **297**: 91-93, 2002.
- Kurasová, I., Čajánek, M., Kalina, J., Urban, O., Špunda, V.: Characterization of acclimation of *Hordeum vulgare* to high irradiation based on different responses of photosynthetic activity and pigment composition. – *Photosynth. Res.* **72**: 71-83, 2002.
- Kurasová, I., Kalina, J., Urban, O., Štroch, M., Špunda, V.: Acclimation of two distinct plant species, spring barley and Norway spruce, to combined effect of various irradiance and CO_2 concentration during cultivation in controlled environment. – *Photosynthetica* **41**: 513-523, 2003.
- Lee, A.I., Thornber, J.P.: Analysis of the pigment stoichiometry of pigment-protein complexes from barley (*Hordeum vulgare*). The xanthophyll cycle intermediates occur mainly in the light-harvesting complexes of photosystem I and photosystem II. – *Plant Physiol.* **107**: 565-574, 1995.
- Lee, H.-Y., Hong, Y.-N., Chow, W.S.: Photoinactivation of photosystem II complexes and photoprotection by non-functional neighbours in *Capsicum annuum* L. leaves. – *Planta* **212**: 332-342, 2001.
- Li, X.-P., Björkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S., Niyogi, K.K.: A pigment-binding protein essential for regulation of photosynthetic light harvesting. – *Nature* **403**: 391-395, 2000.
- Li, X.-P., Gilmore, A.M., Niyogi, K.K.: Molecular and global time-resolved analysis of a *psbS* gene dosage effect on pH- and xanthophyll cycle-dependent nonphotochemical quenching in photosystem II. – *J. biol. Chem.* **277**: 33590-33597, 2002a.
- Li, X.-P., Müller-Moulé, P., Gilmore, A.M., Niyogi, K.K.: PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. – *Proc. nat. Acad. Sci. USA* **99**: 15222-15227, 2002b.
- Li, X.-P., Phippard, A., Pasari, J., Niyogi, K.K.: Structure-function analysis of photosystem II subunit S (PsbS) *in vivo*. – *Funct. Plant Biol.* **29**: 1131-1139, 2002c.
- Logan, B.A., Barker, D.H., Demmig-Adams, B., Adams, W.W., III: Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rainforest. – *Plant Cell Environ.* **19**: 1083-1090, 1996.
- Logan, B.A., Demmig-Adams, B., Adams, W.W., III, Grace, S.C.: Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. – *J. exp. Bot.* **49**: 1869-1879, 1998a.
- Logan, B.A., Grace, S.C., Adams, W.W., III, Demmig-Adams, B.: Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. – *Oecologia* **116**: 9-17, 1998b.
- Lokstein, H., Tian, L., Polle, J.E.W., DellaPenna, D.: Xanthophyll biosynthetic mutants of *Arabidopsis thaliana*: altered nonphotochemical quenching of chlorophyll fluorescence is due to changes in Photosystem II antenna size and stability. – *Biochim. biophys. Acta* **1553**: 309-319, 2002.
- Ma, Y.-Z., Holt, N.E., Li, X.-P., Niyogi, K.K., Fleming, G.R.: Evidence for direct carotenoid involvement in the regulation of photosynthetic light harvesting. – *Proc. nat. Acad. Sci. USA* **100**: 4377-4382, 2003.
- Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – *J. exp. Bot.* **51**: 659-668, 2000.
- McCormac, D.J., Bruce, D., Greenberg, B.M.: State transitions, light-harvesting antenna phosphorylation and light-harvesting antenna migration *in vivo* in the higher plant *Spiriodela oligorrhiza*. – *Biochim. biophys. Acta* **1187**: 301-312, 1994.
- Melis, A.: Photostasis in plants. Mechanisms and regulation. – In: Williams, T.P., Thistle, A.B. (ed.): *Photostasis and Related Phenomena*. Pp. 207-221. Plenum Press, New York 1998.
- 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.
- Morosinotto, T., Caffarri, S., Dall'Osto, L., Bassi, R.: Mechanistic aspects of the xanthophyll dynamics in higher plant thylakoids. – *Physiol. Plant.* **119**: 347-354, 2003.
- Moya, I., Silvestri, M., Vallon, O., Cinque, G., Bassi, R.: Time-resolved fluorescence analysis of the photosystem II antenna proteins in detergent micelles and liposomes. – *Biochemistry* **40**: 12552-12561, 2001.
- Müller, P., Li, X.-P., Niyogi, K.K.: Non-photochemical quenching. A response to excess light energy. – *Plant Physiol.* **125**: 1558-1566, 2001.
- Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M., Shikanai, T.: *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. – *Cell* **110**: 361-371, 2002.
- Munné-Bosch, S., Alegre, L.: The function of tocopherols and tocotrienols in plants. – *Crit. Rev. Plant Sci.* **21**: 31-57, 2002.
- Niyogi, K.K.: Photoprotection revisited: Genetic and molecular approaches. – *Annu. Rev. Plant Physiol. Plant mol. Biol.* **50**: 333-359, 1999.
- Niyogi, K.K.: Safety valves for photosynthesis. – *Curr. Opin. Plant Biol.* **3**: 455-460, 2000.
- Niyogi, K.K., Björkman, O., Grossman, A.R.: *Chlamydomonas* xanthophyll cycle mutants identified by video imaging of chlorophyll fluorescence quenching. – *Plant Cell* **9**: 1369-1380, 1997a.
- Niyogi, K.K., Björkman, O., Grossman, A.R.: The roles of specific xanthophylls in photoprotection. – *Proc. nat. Acad. Sci. USA* **94**: 14162-14167, 1997b.
- Niyogi, K.K., Grossman, A.R., Björkman, O.: *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. – *Plant Cell* **10**: 1121-1134, 1998.
- Niyogi, K.K., Shih, C., Chow, W.S., Pogson, B.J., DellaPenna,

- D., Björkman, O.: Photoprotection in a zeaxanthin- and lutein-deficient double mutant of *Arabidopsis*. – *Photosynth. Res.* **67**: 139-145, 2001.
- Noctor, G., Foyer, C.H.: Ascorbate and glutathione: Keeping active oxygen under control. – *Annu. Rev. Plant Physiol. Plant mol. Biol.* **49**: 249-279, 1998.
- Noguchi, T.: Dual role of triplet localization on the accessory chlorophyll in the photosystem II reaction center: photoprotection and photodamage of the D1 protein. – *Plant Cell Physiol.* **43**: 1112-1116, 2002.
- Nuijs, A.M., Shuvalov, V.A., van Gorkom, H.J., Plijter, J.J., Duysens, L.N.M.: Picosecond absorbance difference spectroscopy on the primary reactions and the antenna-excited states in Photosystem I particles. – *Biochim. biophys. Acta* **850**: 310-318, 1986.
- Öquist, G., Anderson, J.M., McCaffery, S., Chow, W.S.: Mechanistic differences in photoinhibition of sun and shade plants. – *Planta* **188**: 422-431, 1992a.
- Öquist, G., Chow, W.S., Anderson, J.M.: Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. – *Planta* **186**: 450-460, 1992b.
- Ort, D.R., Baker, N.R.: A photoprotective role for O₂ as an alternative electron sink in photosynthesis? – *Curr. Opin. Plant Biol.* **5**: 193-198, 2002.
- Ottander, C., Campbell, D., Öquist, G.: Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. – *Planta* **197**: 176-183, 1995.
- Owens, T.G.: Processing of excitation energy by antenna pigments. – In: Baker, N.R. (ed.): *Photosynthesis and the Environment*. Pp. 1-23. Kluwer Academic Publ., Dordrecht – Boston – London 1996.
- Park, Y.-I., Chow, W.S., Anderson, J.M., Hurry, V.M.: Differential susceptibility of photosystem II to light stress in light-acclimated pea leaves depends on the capacity for photochemical and non-radiative dissipation of light. – *Plant Sci.* **115**: 137-149, 1996.
- Peltier, G., Courmac, L.: Chlororespiration. – *Annu. Rev. Plant Biol.* **53**: 523-550, 2002.
- Pesaresi, P., Sandoa, D., Giuffra, E., Bassi, R.: A single point mutation (E166Q) prevents dicyclohexylcarbodiimide binding to the photosystem II subunit CP29. – *FEBS Lett.* **402**: 151-156, 1997.
- Peterman, E.J.G., Dukker, F.M., van Grondelle, R., van Amerongen, H.: Chlorophyll *a* and carotenoid triplet states in light-harvesting complex II of higher plants. – *Biophys. J.* **69**: 2670-2678, 1995.
- Peterman, E.J.G., Gradinaru, C.C., Calkoen, F., Borst, J.C., van Grondelle, R., van Amerongen, H.: Xanthophylls in light-harvesting complex II of higher plants: Light harvesting and triplet quenching. – *Biochemistry* **36**: 12208-12215, 1997.
- Pfündel, E., Bilger, W.: Regulation and possible function of the violaxanthin cycle. – *Photosynth. Res.* **42**: 89-109, 1994.
- Pogson, B.J., Niyogi, K.K., Björkman, O., DellaPenna, D.: Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. – *Proc. nat. Acad. Sci. USA* **95**: 13324-13329, 1998.
- Pogson, B.J., Rissler, H.M.: Genetic manipulation of carotenoid biosynthesis and photoprotection. – *Phil. Trans. roy. Soc. London B* **355**: 1395-1403, 2000.
- Polívka, T., Herek, J.L., Zigmantas, D., Åkerlund, H.-E., Sundström, V.: Direct observation of the (forbidden) S₁ state in carotenoids. – *Proc. nat. Acad. Sci. USA* **96**: 4914-4917, 1999.
- Polívka, T., Zigmantas, D., Sundström, V., Formaggio, E., Cinque, G., Bassi, R.: Carotenoid S₁ state in a recombinant light-harvesting complex of photosystem II. – *Biochemistry* **41**: 439-450, 2002.
- Pospíšil, P.: Mechanisms of non-photochemical chlorophyll fluorescence quenching in higher plants. – *Photosynthetica* **34**: 343-355, 1997.
- Pursiheimo, S., Mulo, P., Rintamäki, E., Aro, E.-M.: Coregulation of light-harvesting complex II phosphorylation and *lhcb* mRNA accumulation in winter rye. – *Plant J.* **26**: 317-327, 2001.
- Quick, W.P., Stitt, M.: An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. – *Biochim. biophys. Acta* **977**: 287-296, 1989.
- Richter, M., Goss, R., Wagner, B., Holzwarth, A.R.: Characterization of the fast and slow reversible components of non-photochemical quenching in isolated pea thylakoids by picosecond time-resolved chlorophyll fluorescence analysis. – *Biochemistry* **38**: 12718-12726, 1999.
- Rintamäki, E., Salonen, M., Suoranta, U.-M., Carlberg, I., Andersson, B., Aro, E.-M.: Phosphorylation of light-harvesting complex II and photosystem II core proteins shows different irradiance-dependent regulation *in vivo*. Application of phosphothreonine antibodies to analysis of thylakoid phosphoproteins. – *J. biol. Chem.* **272**: 30476-30482, 1997.
- Roháček, K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. – *Photosynthetica* **40**: 13-29, 2002.
- Roháček, K., Barták, M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – *Photosynthetica* **37**: 339-363, 1999.
- Ruban, A.V., Horton, P.: An investigation of the sustained component of nonphotochemical quenching of chlorophyll fluorescence in isolated chloroplasts and leaves of spinach. – *Plant Physiol.* **108**: 721-726, 1995.
- Ruban, A.V., Pascal, A.A., Robert, B., Horton, P.: Activation of zeaxanthin is an obligatory event in the regulation of photosynthetic light harvesting. – *J. biol. Chem.* **277**: 7785-7789, 2002.
- Ruban, A.V., Young, A.J., Horton, P.: Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of the light-harvesting system of photosystem II *in vivo*. – *Plant Physiol.* **102**: 741-750, 1993.
- Sane, P.V., Ivanov, A.G., Hurry, V., Huner, N.P.A., Öquist, G.: Changes in the redox potential of primary and secondary electron-accepting quinones in photosystem II confer increased resistance to photoinhibition in low-temperature-acclimated *Arabidopsis*. – *Plant Physiol.* **132**: 2144-2151, 2003.
- Savitch, L.V., Leonardos, E.D., Krol, M., Jansson, S., Grodzinski, B., Huner, N.P.A., Öquist, G.: Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. – *Plant Cell Environ.* **25**: 761-771, 2002.
- Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – *Photosynth. Res.* **10**: 51-62, 1986.
- Siefermann-Harms, D.: The light-harvesting and protective functions of carotenoids in photosynthetic membranes. – *Physiol. Plant.* **69**: 561-568, 1987.

- Sonoike, K.: Photoinhibition of photosystem I: its physiological significance in the chilling sensitivity of plants. – *Plant Cell Physiol.* **37**: 239-247, 1996.
- Špunda, V., Čajánek, M., Kalina, J., Lachetová, I., Šprtová, M., Marek, M.V.: Mechanistic differences in utilization of absorbed excitation energy within photosynthetic apparatus of Norway spruce induced by the vertical distribution of photosynthetically active radiation through the tree crown. – *Plant Sci.* **133**: 155-165, 1998a.
- Špunda, V., Kalina, J., Čajánek, M., Pavlíčková, H., Marek, M.V.: Long-term exposure of Norway spruce to elevated CO₂ concentration induces changes in photosystem II mimicking an adaptation to increased irradiance. – *J. Plant Physiol.* **152**: 413-419, 1998b.
- Sun, W.-H., Verhoeven, A.S., Bugos, R.C., Yamamoto, H.Y.: Suppression of zeaxanthin formation does not reduce photosynthesis and growth of transgenic tobacco under field conditions. – *Photosynth. Res.* **67**: 41-50, 2001.
- Swiatek, M., Kuras, R., Sokolenko, A., Higgs, D., Olive, J., Cinque, G., Müller, B., Eichacker, L.A., Stern, D.B., Bassi, R., Herrmann, R.G., Wollman, F.-A.: The chloroplast gene *ycf9* encodes a photosystem II (PSII) core subunit, PsbZ, that participates in PSII supramolecular architecture. – *Plant Cell* **13**: 1347-1367, 2001.
- Tardy, F., Havaux, M.: Thylakoid membrane fluidity and thermostability during the operation of the xanthophyll cycle in higher-plant chloroplasts. – *Biochim. biophys. Acta* **1330**: 179-193, 1997.
- Telfer, A.: What is β -carotene doing in the photosystem II reaction centre? – *Phil. Trans. roy. Soc. London B* **357**: 1431-1440, 2002.
- Thayer, S.S., Björkman, O.: Carotenoid distribution and de-epoxidation in thylakoid pigment-protein complexes from cotton leaves and bundle-sheath cells of maize. – *Photosynth. Res.* **33**: 213-225, 1992.
- Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: Close relationship between the state of the xanthophyll cycle pigments and photosystem II efficiency during recovery from winter stress. – *Physiol. Plant.* **96**: 567-576, 1996.
- Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: Two forms of sustained xanthophyll cycle-dependent energy dissipation in overwintering *Euonymus kiautschovicus*. – *Plant Cell Environ.* **21**: 893-903, 1998.
- Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. – *Oecologia* **118**: 277-287, 1999a.
- Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B., Croce, R., Bassi, R.: Xanthophyll cycle pigment localization and dynamics during exposure to low temperatures and light stress in *Vinca major*. – *Plant Physiol.* **120**: 727-737, 1999b.
- Verhoeven, A.S., Bugos, R.C., Yamamoto, H.Y.: Transgenic tobacco with suppressed zeaxanthin formation is susceptible to stress-induced photoinhibition. – *Photosynth. Res.* **67**: 27-39, 2001.
- Wada, M., Kagawa, T., Sato, Y.: Chloroplast movement. – *Annu. Rev. Plant Biol.* **54**: 455-468, 2003.
- Walters, R.G., Horton, P.: Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. – *Photosynth. Res.* **27**: 121-133, 1991.
- Walters, R.G., Ruban, A.V., Horton, P.: Higher plant light-harvesting complexes LHCIIa and LHCIIc are bound by dicyclohexylcarbodiimide during inhibition of energy dissipation. – *Eur. J. Biochem.* **226**: 1063-1069, 1994.
- Walters, R.G., Ruban, A.V., Horton, P.: Identification of proton-active residues in a higher plant light-harvesting complex. – *Proc. nat. Acad. Sci. USA* **93**: 14204-14209, 1996.
- Weis, E., Berry, J.A.: Quantum efficiency of Photosystem II in relation to "energy"-dependent quenching of chlorophyll fluorescence. – *Biochim. biophys. Acta* **894**: 198-208, 1987.
- Wingler, A., Lea, P.J., Quick, W.P., Leegood, R.C.: Photorespiration: metabolic pathways and their role in stress protection. – *Phil. Trans. roy. Soc. London B* **355**: 1517-1529, 2000.
- Wollman, F.-A.: State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. – *EMBO J.* **20**: 3623-3630, 2001.
- Xu, C.C., Jeon, Y.A., Hwang, H.J., Lee, C.-H.: Suppression of zeaxanthin epoxidation by chloroplast phosphatase inhibitors in rice leaves. – *Plant Sci.* **146**: 27-34, 1999.
- Yamamoto, H.Y., Kamite, L.: The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500-nm region. – *Biochim. biophys. Acta* **267**: 538-543, 1972.