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[1]

Prafullachandra Vishnu Sane, Alexander G. Ivanov, Vaughan Hurry, Norman P.A. Huner, and Gunnar Öquist*

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå S–901 87, Sweden (P.V.S., V.H., G.O.); and Biology Department, University of Western Ontario, London, Ontario, Canada N6A 5B7 (A.G.I., N.P.A.H.)

Exposure of control (non-hardened) Arabidopsis leaves for 2 h at high irradiance at 5°C resulted in a 55% decrease in photosystem II (PSII) photochemical efficiency as indicated by F_v/F_m. In contrast, cold-acclimated leaves exposed to the same conditions showed only a 22% decrease in F_v/F_m. Thermoluminescence was used to assess the possible role(s) of PSII recombination events in this differential resistance to photoinhibition. Thermoluminescence measurements of PSII revealed that $S_2Q_A^-$ recombination was shifted to higher temperatures, whereas the characteristic temperature of the $S_2Q_B^-$ recombination was shifted to lower temperatures in cold-acclimated plants. These shifts in recombination temperatures indicate higher activation energy for the $S_2Q_A^-$ redox pair and lower activation energy for the $S_2Q_B^-$ redox pair. This results in an increase in the free-energy gap between $P680^+Q_A^-$ and $P680^+Pheo^-$ and a narrowing of the free energy gap between primary and secondary electron-accepting quinones in PSII electron acceptors. We propose that these effects result in an increased population of reduced primary electron-accepting quinone in PSII, facilitating non-radiative $P680^+Q_A^-$ radical pair recombination. Enhanced reaction center quenching was confirmed using in vivo chlorophyll fluorescence-quenching analysis. The enhanced dissipation of excess light energy within the reaction center of PSII, in part, accounts for the observed increase in resistance to high-light stress in cold-acclimated Arabidopsis plants.

It has been shown previously in winter cereals (Öquist and Huner, 1993) and Arabidopsis (Strand et al., 1997, 1999) that cold acclimation results in an increased capacity for photosynthesis at suboptimal temperatures. This recovery in photosynthetic capacity is closely associated with the posttranslational activation and the selective increase in the expression of enzymes involved in Suc synthesis, with changes in expression and activity of Calvin cycle enzymes (Strand et al., 1997, 1999, 2003; Hurry et al., 2000; Stitt and Hurry, 2002), and with changes in the lipid composition and the content of unsaturated fatty acids of chloroplast membranes (Raison et al., 1982; Hugly and Somerville, 1992; Moon et al., 1995; Routaboul et al., 2000). These changes in photosynthetic capacity and in chloroplast membrane composition protect the photosynthetic apparatus against photoinhibition at low temperatures by allowing increased turnover of the photosynthetic electron transport chain (Hurry et al., 1993, 1995; Huner et al., 1998).

However, results obtained with Scots pine (*Pinus sylvestris*) indicate that cold acclimation can increase the level of photosystem II (PSII) resistance to excessive light directly without any increase in photosynthetic capacity (Krivosheeva et al., 1996). The acquisition of increased tolerance to photoinhibition in cold-acclimated plants has also been ascribed to growth and development under high PSII excitation pressure, i.e. growth conditions that result in a higher reduction state of primary electron-accepting quinone in PSII (Q_A) during steady-state growth at suboptimal temperatures due to an increased reduction of the intersystem electron transport chain (Maxwell et al., 1995; Gray et al., 1996; Huner et al., 1998). It is established that exposure to low nonfreezing temperatures results in an increased PSII excitation pressure, measured as $1 - q_P = \{(Q_A)_{red}/[(Q_A)_{red} + (Q_A)_{ox}]\}$ due to the temperature-dependent decrease in the capacity to use photosynthetic reductants through metabolism. This is a fundamental feature of all taxonomic groups of photosynthetic organisms (Öquist and Huner, 1993; Öquist et al., 1993; Maxwell et al., 1995; Gray et al., 1996; Huner et al., 1998). Thus, although photosynthetic acclimation to low temperature may be regulated, in part, by cellular metabolic

[1] This work was supported by the Swedish Foundation for International Cooperation in Research and Higher Education, by the Swedish Research Council, and by the Natural Science and Engineering Research Council of Canada.

* Corresponding author; e-mail gunnar.oquist@plantphys.umu.se; fax 46–90–7866676.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.103.022939.

status (Hurry et al., 2000; Stitt and Hurry, 2002) and/or redox status of a specific component(s) of the photosynthetic electron transport chain (Maxwell et al., 1995; Gray et al., 1996; Huner et al., 1998), it appears that certain developmental alterations to PSII may also enhance its resistance to excess light (Huner et al., 1998).

There is a consensus that non-photochemical quenching of excess absorbed light in the antenna, via the induction of the xanthophyll cycle, is an important mechanism to protect the photosynthetic apparatus from photodamage (Demmig-Adams and Adams, 1992; Horton et al., 1996; Niyogi, 1999). However, there have also been suggestions that a component of non-photochemical quenching exists in Arabidopsis that is independent of the xanthophyll cycle (Niyogi et al., 1998). Although different potential mechanisms of photosynthetic adjustments to high PSII excitation pressure have been discussed (for review, see Huner et al., 1998), the exact role of the acceptor side of PSII in acclimation of the photosynthetic apparatus has not been evaluated directly. Because PSII, especially its acceptor side, has generally been considered to be the primary target for photoinhibition of photosynthesis (Powles, 1984; Krause, 1988; Öquist et al., 1992; Aro et al., 1993), we have used thermoluminescence (TL) measurements (Sane and Rutherford, 1986) to evaluate more precisely the charge recombination events between the acceptor and donor sides of PSII during acclimation of Arabidopsis plants to low temperature.

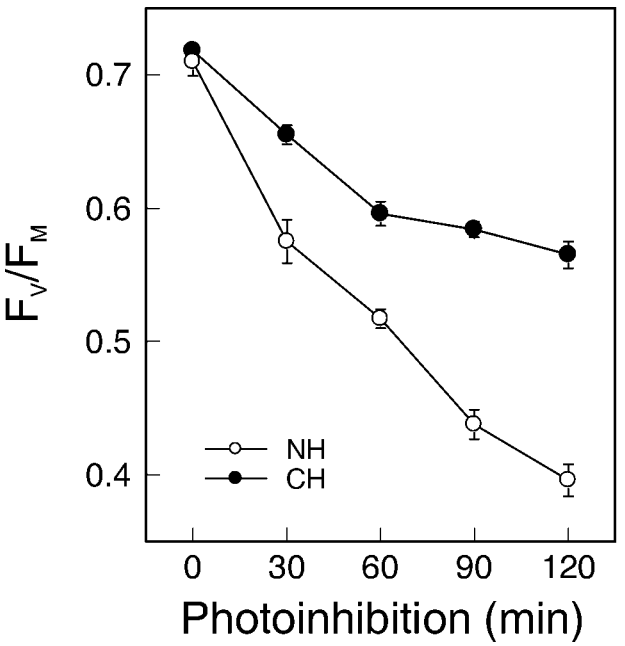

Figure 1. The effects of high-light treatment (600 μmol photons m^{-2} s^{-1}) at 5°C on the photochemical efficiency of PSII measured as F_v/F_m in control (NH; ○) and cold-acclimated (CH; ●) Arabidopsis leaves. All measurements were performed at the corresponding growth temperature. The data are presented as a percentage of F_v/F_m values in dark-adapted (15 min) samples of nontreated leaves. Mean values ± SE were calculated from five to seven measurements in three to five independent experiments.

RESULTS

Exposure of control non-hardened (NH) Arabidopsis leaves to high irradiance at 5°C for 2 h resulted in a gradual decrease of the maximal PSII photochemical efficiency as shown by a 55% decrease in the F_v/F_m values relative to control (nontreated) leaves (Fig. 1). Similar responses have been reported in earlier studies for Arabidopsis leaves exposed to high irradiance at warm (Russell et al., 1995: Hurry et al., 1997) and at chilling temperatures (Havaux and Kloppstech, 2001). In contrast, exposure of cold-acclimated leaves to high irradiance caused much less reduction of PSII efficiency during the same time interval (Fig. 1).

The possibility of cold-induced alterations to PSII primary photochemistry was addressed by TL measurements for direct estimation and comparison of the redox properties of PSII (Sane and Rutherford, 1986) in control (NH) and cold-acclimated (cold-hardened [CH]) Arabidopsis plants. Typical TL curves for NH and CH Arabidopsis leaves after exposure to two consecutive flashes of saturating white light in the presence and absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) are presented in Figure 2. Deconvolution of the TL glow signal of NH control Arabidopsis leaves yielded three peaks with characteristic temperature of maximum thermoluminescence emission (T_M) of −12°C, 38°C, and 54°C, respectively (Fig. 2A). The major peak around 38°C accounted for about 68% of the total TL emission. The peak around 54°C accounted for most of the remaining luminescence, whereas the peak appearing near −12°C contributed about 5% of the total luminescence (Table I). It has been demonstrated earlier that the major TL band(s) produced after two saturating flashes is related to both $S_2Q_B^-$ and $S_3Q_B^-$ recombinations (Meyers et al., 1993; Mäenpää et al., 1995). More precise identification of the recombining redox species corresponding to each of the peaks was achieved by determining the glow curve patterns in the presence of DCMU. As expected, in the presence of DCMU, the overall TL emission was strongly reduced, the peak appearing at 38°C was lost, and a new peak with a T_M of −18°C appeared (Fig. 2C). Because DCMU specifically inhibits electron transport between Q_A and the secondary electron-accepting quinone in PSII (Q_B) and concomitant conversion of $S_2Q_B^-$ to $S_2Q_A^-$ (Inoue, 1996), the data presented above clearly show that the peak appearing around 38°C in the absence of DCMU (Fig. 2A) originates from $S_2Q_B^-$ recombination, whereas the appearance of DCMU-resistant peak around −18°C (Fig. 2C) is ascribed to the $S_2Q_A^-$ recombination (Inoue, 1996; Vass and Govindjee, 1996). The TL peaks with characteristic T_M above 50°C (Fig. 2, A–D)

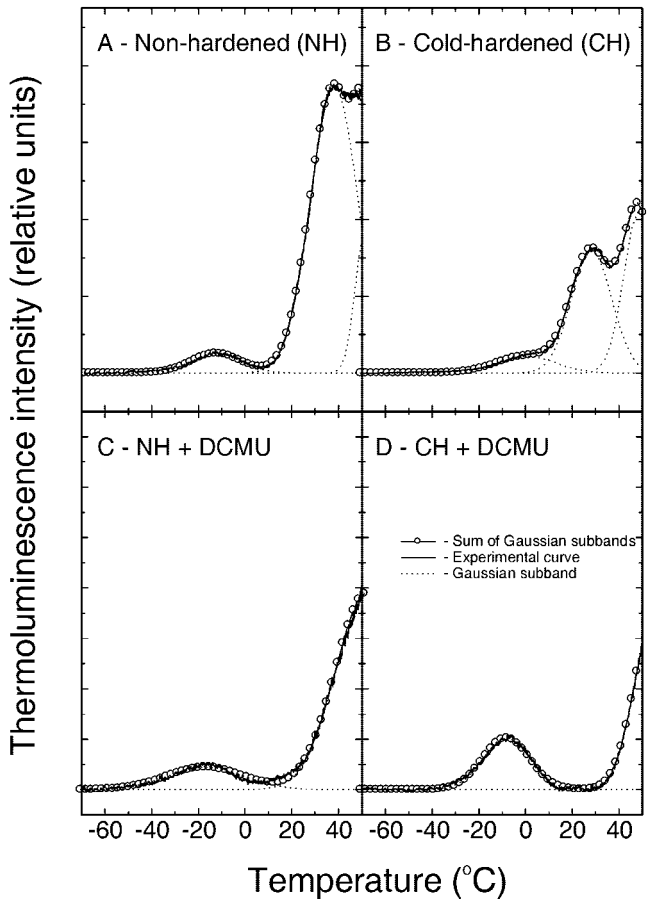

Figure 2. TL glow curves and mathematical decomposition in subbands of control NH (25°C; A and C) and cold-acclimated (5°C; B and D) Arabidopsis leaves after illumination with two single-turnover flashes of white saturating light. A and B, Glow curves of control NH (25°C) and cold-acclimated (5°C) leaves in the absence and in the presence of DCMU (C and D), respectively. The presented glow curves are averages from five to seven measurements in three independent experiments.

could be assigned to the C band (Vass and Govindjee, 1996).

A typical TL glow curve pattern of CH Arabidopsis leaves is presented in Figure 2B. Apart from the lower overall TL emission compared with that observed for NH leaves, the glow curve exhibited a major peak appearing at 28°C, which contributed about 46% of the total luminescence; a minor peak at about 0°C, which contributed about 10% of the total TL emission; and a high-temperature C-band at 48°C (Table I). As expected, addition of DCMU caused a further reduction of the total TL emission and a loss of the peak at 28°C. This was accompanied by the appearance of a new peak centered at −8°C (Fig. 2D). The major peaks appearing in the absence of DCMU are attributed to the $S_2Q_B^-$ recombination (B-band), whereas those appearing in the presence of DCMU are attributed to the $S_2Q_A^-$ recombination (Q-band; Sane and Rutherford, 1986; Vass and Govindjee, 1996). The characteristic T_M of $S_2Q_A^-$ and $S_2Q_B^-$ recombinations in NH leaves appeared at −17.6°C and 37.7°C, respectively (Table I). In contrast, CH leaves exhibited characteristic T_M at −8.1°C and 28.3°C for the $S_2Q_A^-$ and $S_2Q_B^-$ recombinations, respectively. The temperature gap between the $S_2Q_A^-$ and $S_2Q_B^-$ peaks is thus narrowed to about 36°C in CH plants as compared with a gap of about 55°C in NH plants. The shift of the $S_2Q_B^-$ peak to lower temperatures suggests a significant decrease in the activation energy for the $S_2Q_B^-$ radical pair in CH plants. The significant shift (about 10°C) of T_M for the $S_2Q_A^-$ emission band to higher temperature in CH leaves (Table I) suggests an increased activation energy and stabilization of the $S_2Q_A^-$ charge pair (Krieger-Liszkay and Rutherford, 1998) compared with that in NH Arabidopsis.

The shift in the $S_2Q_A^-$ and $S_2Q_B^-$ peak temperatures was examined in a series of experiments in which NH plants were transferred to 5°C (Fig. 3A). These data clearly show that whereas the characteristic T_M of $S_2Q_B^-$ peak gradually decreased, the T_M of $S_2Q_A^-$ shifted to higher temperatures. Thus, the initial gap of 55°C between $S_2Q_A^-$ and $S_2Q_B^-$ in NH Arabidopsis was narrowed to about 36°C over a period of 4 weeks. In addition, the relative TL yield measured as the integrated area under the glow curves sharply decreased by 40% after a 7-d exposure of control NH plants to 5°C, followed by a gradual reduction of the TL emission. After 24 d at low temperature, the shifted NH plants exhibited overall TL luminescence close to that of fully CH plants (Fig. 3B). This effect could be due to differential reabsorption of the luminescence caused by different chlorophyll (Chl) concentrations. However, because the changes in Chl content per unit leaf area during the exposure to low temperature are minimal (Strand et al., 1999, 2003), we believe that the observed changes in the overall TL emission reflect differences in the deactivation pathways rather than the differences in Chl content.

The decay of variable Chl fluorescence monitoring the oxidation of Q_A^- after a single saturating flash of dark-adapted control NH Arabidopsis leaves (Fig. 4A) exhibited complex kinetics that could be resolved into three different decay components (Table II) similar to those reported by others (Cao and Govindjee, 1990; Govindjee et al., 1992; Ivanov et al., 2001). The estimated decay half-times for the first two phases (fast and medium) of Q_A^- re-oxidation in control NH Arabidopsis leaves were 201 and 1,350 μs, accounting for 43% and 24% of the decay, respectively. The slow phase, presumably associated with a back reaction of Q_A^- with the S_2 states in centers in which Q_A is poorly connected to Q_B and the plastoquinone pool (Etienne et al., 1990), exhibited a half-time in the 0.5- to 2-s range and accounted for the remaining 33% of the decay. In contrast, the fast and the medium components of the Q_A^- re-oxidation in CH Arabidopsis were significantly slower than observed in NH leaves

Table I. *Peak emission temperatures (T_M) and contribution of different glow peaks to the total thermoluminescence in control (non-hardened) and cold-acclimated Arabidopsis leaves*

The contribution of glow peaks represented by characteristic Gaussian sub-bands was estimated by a nonlinear least-squares fitting of the experimental glow curves obtained after illumination with two single turnover flashes. The data are represented as a percentage of the total area (A, %). Mean values ± SE were calculated from three to five independent experiments. n.d., Non-detectable. The measurements were performed in the absence and presence of 20 μM DCMU.

Sample	Control		+DCMU	
	T_M	A	T_M	A
	°C	%	°C	%
Non-hardened	−12.0 ± 0.06	5.1 ± 0.1	−17.6 ± 0.08	7.9 ± 0.1
	37.7 ± 0.03	67.8 ± 0.1	n.d.	n.d.
	53.9 ± 0.06	27.1 ± 0.1	58.7 ± 0.08	92.1 ± 0.2
Cold acclimated	0.3 ± 0.1	9.1 ± 0.1	−8.1 ± 0.02	25.5 ± 0.1
	28.3 ± 0.02	45.6 ± 0.1	n.d.	n.d.
	48.6 ± 0.06	45.3 ± 0.2	55.3 ± 0.07	74.5 ± 0.2

(Fig. 4B; Table II). The medium phase accounted for 37% of the fluorescence decay, whereas the fast component (42%) was comparable with that in control leaves.

Possible differences between NH and CH plants in the mode of light energy dissipation were studied using Chl fluorescence-quenching analysis. Quenching was achieved by applying actinic white light illumination of different photon flux densities, and the experimental data for the quenching of basal F_o fluorescence (q_O) were plotted against non-photochemical (q_N) quenching (Fig. 5). If quenching of F_o is a reliable indicator of energy dissipation in the antenna complexes (Rees et al., 1990; Bukhov et al., 2001), the increase of q_O in relation to q_N implies that the antenna quenching in NH Arabidopsis leaves is substantial. In contrast, the q_O versus q_N relationship of quenching in CH plants demonstrates that the increase of q_O is very limited within a broad range of q_N and became evident only at very high irradiance (Fig. 5). This suggests that an additional energy dissipation mechanism, which is independent of antenna quenching, is more prominent in CH than NH Arabidopsis leaves.

DISCUSSION

The differences in the TL glow curves between control and CH plants clearly suggest major alterations in the redox properties of the acceptor side of PSII during cold acclimation in Arabidopsis. The flash-induced TL bands in cold-acclimated plants associated with $S_2Q_A^-$ and $S_2Q_B^-$ recombinations showed shifts in the characteristic peak T_M with the Q_A-associated peak appearing at higher temperatures, whereas the Q_B-associated peak was shifted to lower temperatures compared with control Arabidopsis leaves (Figs. 2 and 3A; Table I). These changes imply substantial changes in the activation energies associated with detrapping of the electron from reduced Q_A and Q_B. Because the activation energies are directly related to the redox potentials of the participating species (deVault and Govindjee, 1990), narrowing the gap between the characteristic T_M for Q_A and Q_B by 19°C reflects a narrowing of the redox potential gap between Q_A and Q_B as a result of cold acclimation.

If we consider that both the Q_A- and Q_B-related TL peaks from cold-acclimated plants showed shifts in their characteristic temperatures, it seems reasonable to suggest that the redox potentials of both acceptors

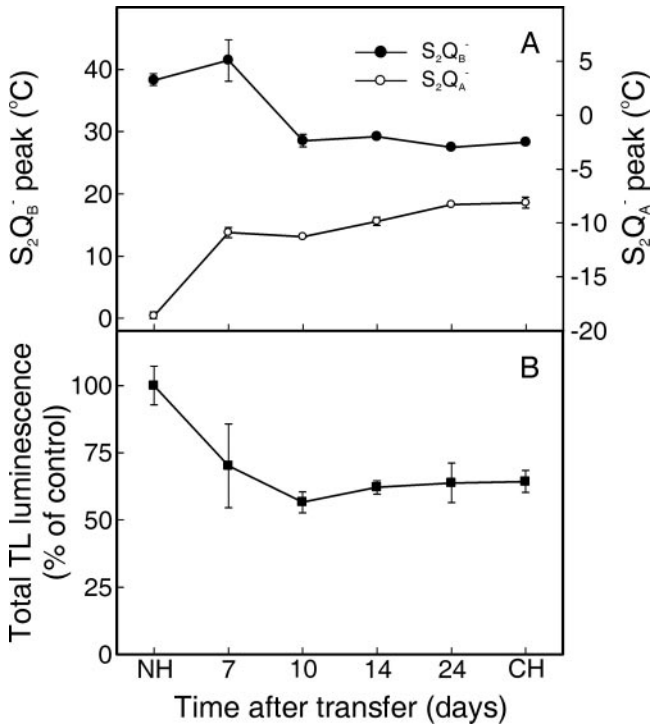

Figure 3. A, Time course of the characteristic T_M of $S_2Q_B^-$ and $S_2Q_A^-$ peaks in Arabidopsis leaves during the temperature shift of control (NH) plants from the growth temperature of 23°C to 5°C. The peak positions were estimated by decomposition analysis of the experimental curves in control and in DCMU-treated leaves. B, Relative TL yield measured as the total area under the experimental glow curves. The mean values ± SE were calculated from six to eight measurements in three independent experiments. NH, Control plants; CH, fully cold-acclimated plants.

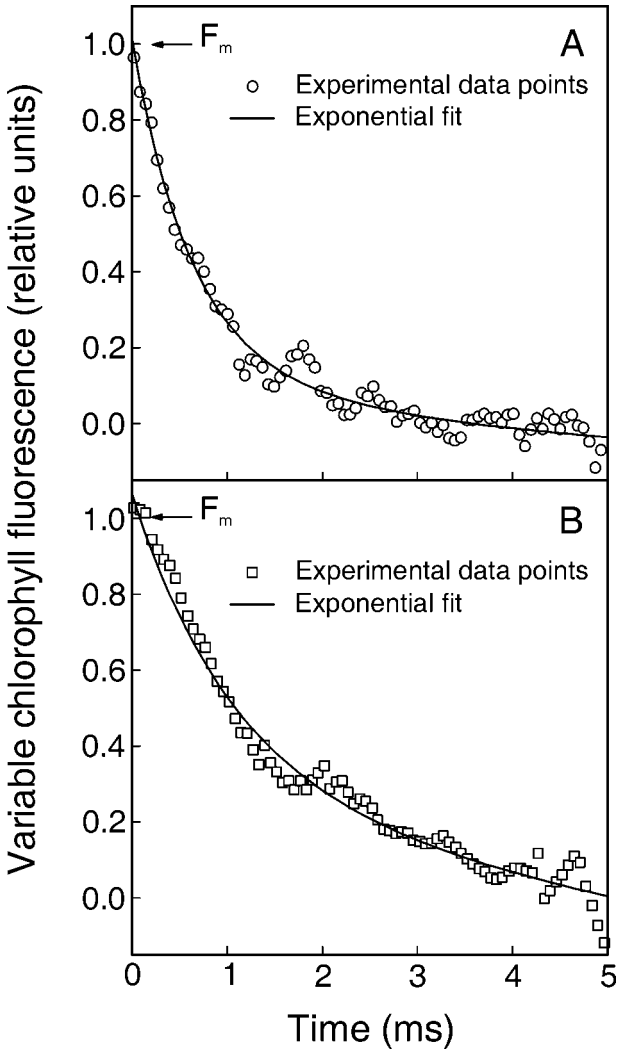

Figure 4. Chl fluorescence decay kinetics after single-turnover flash illumination in control (A) and cold-acclimated (B) Arabidopsis leaves. Drawn lines are fits for the experimental data points. Experimental fluorescence curves were normalized to the corresponding F_m values and represent averages from eight to nine separate experiments.

may have changed. In addition, the possibility that the redox characteristics of the oxidizing species (S_2 and S_3 in this case) participating in the recombination reactions may also have changed cannot be ruled out.

Table II. *Kinetic parameters of variable fluorescence yield decay in control and cold-acclimated Arabidopsis leaves*

Exponential analysis yielded triphasic kinetics with different half times ($\tau_{1/2}$) and amplitude (A). Mean values ± SE were calculated from eight to 10 recordings.

Parameters	Control	Cold Acclimated
τ_{fast} (μs)	201 ± 19	340 ± 67
τ_{int} (μs)	1,350 ± 56	4,990 ± 363
A_{fast} (%)	43.3	42.0
A_{int} (%)	23.9	36.7
χ^2	0.001	0.002

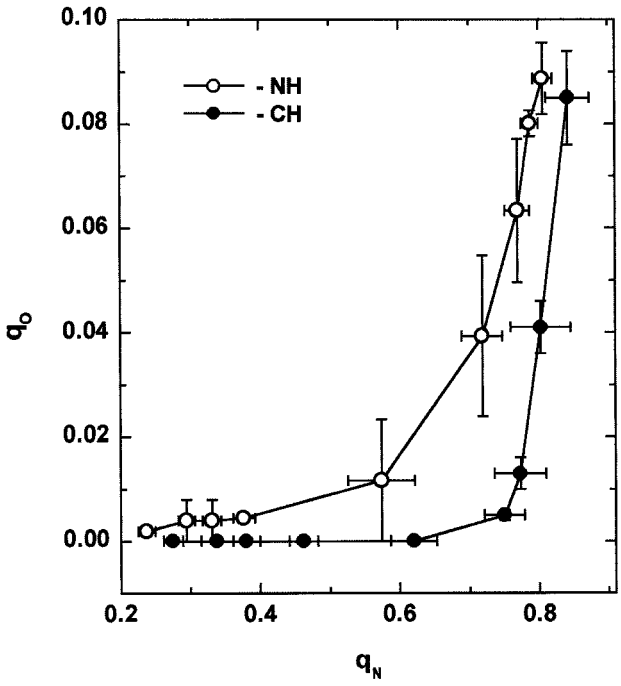

Figure 5. q_N versus q_O Chl fluorescence quenching measured at different actinic light intensities in NH (white symbols) and cold-acclimated (black symbols) Arabidopsis leaves. Mean values ± SE were calculated from three to four independent experiments.

However, if there had been a change in the redox characteristics of the S_2/S_3 states, the change should have been in the same direction. Our observations indicate that the $S_2Q_A^-$ peak was shifted toward higher temperature, whereas the $S_2Q_B^-$ peak shifted toward a lower temperature. Thus, we believe that the redox characteristics of the oxidizing species are not changing significantly during cold acclimation and that the changes in TL peak temperatures reflect changes mainly in the redox characteristics of Q_A and/or Q_B.

The high-temperature shift in the T_M of $S_2Q_A^-$, corresponding to increased activation energy of Q_A/Q_A^-, would increase the free energy gap between $P680^+Q_A^-$ and $P680^+Pheo^-$. This could cause stabilization of $S_2Q_A^-$ and decrease the probability for the back reaction through $P680^+Pheo^-$ (Minagawa et al., 1999; Vavilin and Vermass, 2000). Moreover, the preferential localization of the electron on Q_A in cold-acclimated Arabidopsis could also result from a change in the redox potential of Q_B. Lowering the redox potential of Q_B will narrow the gap between the redox potentials between Q_A and Q_B even further and will decrease the probability for forward electron transfer between the two quinone acceptors by shifting the redox equilibrium between $Q_A^-Q_B$ and $Q_AQ_B^-$ toward $Q_A^-Q_B$ (Fig. 6; Minagawa et al., 1999). The retention of electrons preferentially on Q_A through a modification of the redox potentials of Q_A and Q_B in opposite directions may cause the reoxidation of Q_A^- by forward and/or back reaction to

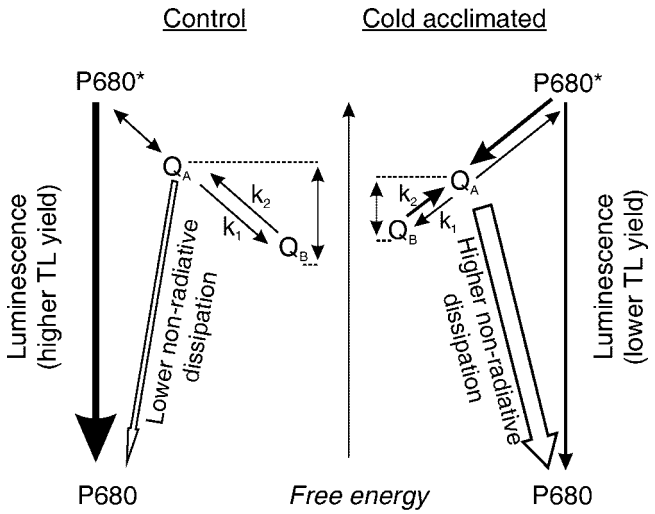

Figure 6. Schematic diagram of the free energy levels explaining the differences in radiative versus non-radiative energy dissipation pathways in control (A) and cold-acclimated (B) Arabidopsis leaves. In control leaves, radiative energy dissipation pathway characterized by higher TL yield is predominant and probably involves the back reaction via the $P680^+Pheo^-$ radical pair. In cold-acclimated leaves, the increased free energy gap between $P680^+$ and Q_A^- would decrease the probability for a charge recombination pathway involving $P680^+Pheo^-$ and will cause stabilization of $S_2Q_A^-$ pair. In addition, shifting the redox potential of Q_B toward Q_A favors the k2 rate constant and also results in increased steady-state proportion of reduced Q_A. It is proposed that this will increase the probability for direct recombination of Q_A^- with $P680^+$ via non-radiative interaction resulting in low-TL yield without generating Chl triplet. In both types of plants, the radiative charge recombination occurs, but is proportionally less in cold-acclimated plants. The model is based on the assumption that redox properties of the donor side were not modified during cold acclimation.

become more difficult (Mäenpää et al., 1995). In fact, slower reoxidation kinetics of Q_A^- were found in cold-acclimated Arabidopsis leaves (Fig. 4; Table II). This would ensure that the Q_B site remains occupied by a quinone, which would protect PSII from photoinhibition and D1 degradation. Supporting evidence for this argument comes from experiments in which addition of DCMU had a protective effect on D1 turnover under photoinhibitory conditions (Komenda and Masojidek, 1998). When the Q_B site is occupied in the presence of DCMU and Q_A is in a reduced state, PSII shows increased resistance to photoinhibition.

A possible back reaction between the reduced Q_A and $P680^+$ has been suggested previously (Prasil et al., 1996; Krieger-Liszkay and Rutherford, 1998), and this may be enhanced when Q_A remains reduced (Vavilin and Vermass, 2000). The accumulation of Q_A^- has been shown to inhibit the formation of the radical pair $P680^+Pheo^-$ thus preventing P680 triplet formation (Schatz et al., 1988; Vass et al., 1992). In addition, it has been suggested that there is a non-radiative pathway of charge recombination between Q_A^- and the donor side of PSII (Briantais et al., 1979; Weis and Berry, 1987; Vavilin and Vermass, 2000). Such a pathway would increase the probability for non-radiative dissipation of excitation energy within the reaction center of PSII (Weis and Berry, 1987; Bukhov et al., 2001). The reduction of Q_A has been suggested to be a major requirement for efficient reaction center quenching (Bukhov et al., 2001). In this regard, it is important to note that acclimation to low temperatures is strongly correlated with an increased proportion of reduced Q_A at the given growth temperature (Huner et al., 1998). Moreover, our recent data also indicated an increased proportion of Q_A^- (measured as 1-qP) in cold-acclimated Arabidopsis (Savitch et al., 2001). Hence, it seems very likely that the increased population of Q_A^- due to the altered redox potential of Q_A and Q_B during the shift and acclimation to low temperature in Arabidopsis may enhance the dissipation of excess light within the reaction center of PSII via non-radiative $P680^+Q_A^-$ recombination, protecting the Q_B site from excessive excitation pressure. Similar trends were reported for both winter Scots pine (Ivanov et al., 2001) and low-temperature-stressed *Synechococcus* sp. (Sane et al., 2002).

If the non-radiative reaction center energy dissipation pathway is enhanced, a substantial reduction in the overall TL yield should be expected in CH Arabidopsis. The quantitative analysis of the TL glow curves showed a 40% lower TL yield in CH plants and a time-dependent decline of total TL yields during the shift of control NH plants from 23°C to 5°C (Fig. 3B). Analysis of antenna-based energy dissipation measured as q_O versus q_N has been used to assess the relative contribution of quenching originating from antenna complexes versus reaction center quenching (Rees et al., 1990; Bukhov et al., 2001). Using the model of fluorescence quenching proposed by Bukhov et al. (2001), the very limited increase of q_O relative to q_N values between 0.2 and 0.6 in CH Arabidopsis leaves (Fig. 5) indicates that a significant capacity for energy dissipation exists that is independent of antenna quenching in CH Arabidopsis. In support of this conclusion, the *npq1* and *npq4* mutants of *Chlamydomonas reinhardtii* are able to acclimate to high light (Niyogi et al., 1997; Niyogi, 1999), and long-term acclimation of the Arabidopsis *npq1* mutant to chilling stress in the light proceeds without increased photodamage of the photosynthetic apparatus (Havaux and Kloppstech 2001). This confirms an earlier suggestion that a component of non-photochemical quenching exists in Arabidopsis that is independent of the xanthophyll cycle (Niyogi et al., 1998). These data clearly indicate that defects in *npq* resulting in inhibition of non-photochemical quenching can be compensated by alternative mechanisms for dissipating the excess light energy. We suggest that PSII reaction center quenching fulfils this role.

In summary, the results presented in this study demonstrate that acclimation of Arabidopsis plants to low (5°C) temperature is associated with major

changes within the acceptor side of PSII involving the redox potentials of Q_A and Q_B. We suggest that increasing the free energy gap between $P680^+$ and Q_A^- and narrowing the gap between the redox potential of Q_A and Q_B in CH Arabidopsis plants (Fig. 6) result in stabilization of $S_2Q_A^-$. We propose that the increased population of Q_A^- facilitates its back reaction with $P680^+$ via non-radiative recombination, enhancing the dissipation of excess light energy within the reaction center of PSII. This phenomenon together with the recovery of photosynthetic capacity (Savitch et al., 2001) may explain the increased resistance of CH Arabidopsis plants to low-temperature photoinhibition.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis ecotype Col-0 seeds were germinated under controlled environment conditions with an irradiance of 150 μmol m^{-2} s^{-1}, a 23°C/18°C (day/night) temperature regime, and an 8-h photoperiod. After 20 d, when the leaves had developed into the fully mature source leaves, some plants were shifted to a 5°C/5°C temperature regime with the same photoperiod and irradiance. Plants were considered cold acclimated when a second rosette of leaves had developed and fully expanded at 5°C. All measurements were made on fully expanded leaves of either warm-grown control (23°C) or cold-acclimated (5°C) plants.

TL Measurements

TL measurements of Arabidopsis leaves were performed on a personal computer-based TL data acquisition and analysis system as described earlier (Ivanov et al., 2001). A photomultiplier tube (Hamamatsu R943-02, Hamamatsu Photonics K.K., Shizuoka-ken, Japan) equipped with a photomultiplier power supply (model PS-302, EG&G Electro Optics, Salem, MA) and a preamplifier (model C1556-03) was used as a radiation measuring set. Decomposition analysis of the TL glow curves was carried out by a nonlinear, least-squares algorithm that minimizes the chi-square function using a Microcal Origin v6.0 software package (Microcal Software, Northampton, MA). The nomenclature of Vass and Govindjee (1996) was used for characterization of the TL glow peaks. Experiments were usually performed at a 0.6°C s^{-1} heating rate.

A flash lamp assembly (Type FX200, EG&G Electro Optics) was used to expose the sample to two single-turnover flashes (2.5-μs half-band with 10-Hz frequency). For this purpose, the leaves were first dark adapted for 10 min at 20°C and then cooled to 0°C before exposure to the flashes. After the flash exposure, the sample was quickly cooled in liquid nitrogen. For $S_2Q_A^-$ recombination studies, leaves were vacuum infiltrated with DCMU (20 μM) in darkness before the flash illumination.

Modulated Chl Fluorescence

Chl *a* fluorescence of a dark-adapted (30 min) leaves from NH and cold-acclimated Arabidopsis plants was measured using a PAM 101 Chl fluorescence measuring system (Heinz Walz GmbH, Effeltrich, Germany) under ambient CO_2 conditions as described by Ivanov et al. (1998). Instantaneous (dark) Chl fluorescence at open PSII centers (F_o) was excited by a nonactinic modulated measuring beam (650 nm, 0.12 μmol m^{-2} s^{-1}) at 1.6 kHz in the dark and 100 kHz in the light. Maximum fluorescence at closed PSII centers (F_m) was induced by saturating white light pulses (800 ms, 2800 μmol m^{-2} s^{-1}) provided by a Schott lamp (KL 1500, Schott Glaswerke, Mainz, Germany) and controlled from a Walz PAM 103 Trigger Control Unit. All measurements were performed at the corresponding growth temperature. q_N and q_O Chl fluorescence-quenching parameters were calculated using the procedure described by Quick and Stitt (1989). The fluorescence characteristics were evaluated when the steady-state F_s level was reached.

Photoinhibition Treatment

For high-light treatment, leaves from NH and cold-acclimated Arabidopsis plants were exposed to photosynthetically active radiation of 600 μmol photons m^{-2} s^{-1} white light at 5°C.

Chl *a* Fluorescence Decays

The re-oxidation kinetics of Q_A^- were measured as the decay of Chl *a* fluorescence using a pulse-amplitude modulated fluorimeter as described earlier (Ivanov et al., 2001). Saturating single-turnover flashes obtained from an XST 103 xenon discharge lamp connected to a PAM 103 unit (Heinz Walz) were used to convert all Q_A to Q_A^-. The variable fluorescence decay, reflecting the re-oxidation of Q_A^-, was detected at 20-μs resolution. The signals were recorded using an oscilloscope card (PC-SCOPE T6420 v2.43x, Intelligente Messtechnik GmbH, Backnang, Germany) installed in an IBM-compatible personal computer. Data from at least 10 recordings were averaged. Final curve fitting was performed by a nonlinear data analysis using a Microcal Origin v6.0 software package (Microcal Software).

Received March 4, 2003; returned for revision March 9, 2003; accepted April 29, 2003.

LITERATURE CITED

Aro E-M, Virgin I, Andersson B (1993) Photoinhibition of photosystem II: inactivation, protein damage and turnover. Biochim Biophys Acta **1143:** 113–134

Briantais J-M, Vernotte C, Picaud M, Krause GH (1979) A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. Biochim Biophys Acta **548:** 128–138

Bukhov NG, Heber U, Wiese C, Shuvalov VA (2001) 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

Cao J, Govindjee (1990) Chlorophyll *a* fluorescence transients as an indicator of active and inactive photosystem II in thylakoid membranes. Biochim Biophys Acta **1015:** 180–188

Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol **43:** 599–626

deVault D, Govindjee (1990) Photosynthetic glow peaks and their relationship with the free energy changes. Photosynth Res **24:** 175–181

Etienne A-L, Ducruet J-M, Ajilani G, Vernotte C (1990) Comparative studies on electron transfer in photosystem II of herbicide resistant mutants from different organisms. Biochim Biophys Acta **1015:** 435–440

Govindjee, Eggenberg P, Pfister K, Strasser RJ (1992) Chlorophyll *a* fluorescence decay in herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii* and the formate effects. Biochim Biophys Acta **1101:** 353–358

Gray GR, Savitch LV, Ivanov AG, Huner NPA (1996) Photosystem II excitation pressure and development of resistance to photoinhibition: II. Adjustment of photosynthetic capacity in winter wheat and winter rye. Plant Physiol **110:** 61–71

Havaux M, Kloppstech K (2001) The protective functions of carotenoids and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta **213:** 953–966

Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 655–684

Hugly S, Somerville C (1992) A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature. Plant Physiol **99:** 197–202

Huner NPA, Öquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. Trends Plant Sci **3:** 224–230

Hurry V, Anderson JM, Chow WS, Osmond CB (1997) Accumulation of zeaxanthin in abscisic acid-deficient mutants of Arabidopsis does not affect chlorophyll fluorescence quenching or sensitivity to photoinhibition in vivo. Plant Physiol **113:** 639–648

Hurry V, Strand Å, Furbank R, Stitt M (2000) The role of inorganic phosphate in the development of freezing tolerance and the acclimatization of photosynthesis to low temperature is revealed by the pho mutants of *Arabidopsis thaliana*. Plant J **24:** 383–396

Hurry VM, Gardeström P, Öquist G (1993) Reduced sensitivity to photoinhibition following frost-hardening of winter rye is due to increased phosphate availability. Planta **190:** 484–490

Hurry VM, Strand Å, Tobiæson M, Gardeström P, Öquist G (1995) Cold hardening of spring and winter-wheat and rape results in differential-effects on growth, carbon metabolism, and carbohydrate content. Plant Physiol **109:** 697–706

Inoue Y (1996) Photosynthetic thermoluminescence as a simple probe of photosystem II electron transport. *In* J Amesz, A Hoff, eds, Biophysical Techniques in Photosynthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 93–107

Ivanov AG, Morgan RM, Gray GR, Velitchkova MY, Huner NPA (1998) Temperature/light dependent development of selective resistance to photoinhibition of photosystem I. FEBS Lett **430:** 288–292

Ivanov AG, Sane PV, Zeinalov Y, Malmberg G, Gardeström P, Huner NPA, Öquist G (2001) Photosynthetic electron transport adjustments in overwintering Scots pine (*Pinus sylvestris* L.). Planta **213:** 575–585

Komenda J, Masojidek J (1998) The effect of photosystem II inhibitors DCMU and BNT on the light-induced D1 turnover in two cyanobacterial strains *Synechocystis* PCC 6803 and *Synechococcus* PCC 7942. Photosynth Res **57:** 193–202

Krause GH (1988) Photoinhibition of photosynthesis: an evaluation of damaging and protective mechanisms. Physiol Plant **74:** 566–574

Krieger-Liszkay A, Rutherford AW (1998) Influence of herbicide binding on the redox potential of the quinone acceptor in photosystem II: relevance to photodamage and phototoxicity. Biochemistry **37:** 17339–17344

Krivosheeva A, Tao D-L, Ottander C, Wingsle G, Dube SL, Öquist G (1996) Cold acclimation and photoinhibition of photosynthesis in Scots pine. Planta **200:** 296–305

Mäenpää P, Miranda T, Tyystjarvi E, Govindjee, Ducruet J-M, Kirilovsky D (1995) A mutation in the D-*de* loop of D1 modifies the stability of the $S_2Q_A^-$ and $S_2Q_B^-$ states in photosystem II. Plant Physiol **107:** 187–197

Maxwell DP, Falk S, Huner NPA (1995) Photosystem II excitation pressure and development of resistance to photoinhibition: I. Light-harvesting complex II abundance and zeaxanthin content in *Chlorella vulgaris*. Plant Physiol **107:** 687–694

Meyers SR, Dubbs JM, Vass I, Hideg E, Nagy L, Barber J (1993) Further characterization of the *psb*H locus of *Synechocystis* sp. PCC 6803: inactivation of *psb*H impairs Q_A to Q_B electron transport in photosystem 2. Biochemistry **32:** 1454–1465

Minagawa J, Narusaka Y, Inoue Y, Satoh K (1999) Electron transfer between Q_A and Q_B in photosystem II is thermodynamically perturbed in phototolerant mutants of *Synechocystis sp*. PCC 6803. Biochemistry **38:** 770–775

Moon BY, Higashi S, Gombos Z, Murata N (1995) Unsaturation of the membrane lipids of chloroplasts stabilizes the photosynthetic machinery against low-temperature photoinhibition in transgenic tobacco plants. Proc Natl Acad Sci USA **92:** 6219–6223

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approach. Annu Rev Plant Physiol Plant Mol Biol **50:** 333–359

Niyogi KK, Bjorkman O, Grossman AR (1997) The roles of specific xanthophylls in photoprotection. Proc Natl Acad Sci USA **94:** 14162–14167

Niyogi KK, Grossman AR, Bjorkman O (1998) Arabidopsis mutants define a central role for xanthophyll cycle in the regulation of photosynthetic energy conversion. Plant Cell **10:** 1121–1134

Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosynthesis. Planta **186:** 450–460

Öquist G, Huner NPA (1993) Cold hardening induced resistance to photoinhibition in winter rye is dependent upon increase capacity for photosynthesis. Planta **189:** 150–156

Öquist G, Hurry VM, Huner NPA (1993) The temperature dependence of the redox state of Q_A and the susceptibility of photosynthesis to photoinhibition. Plant Physiol Biochem **31:** 683–691

Powles SB (1984) Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol **35:** 15–44

Prasil O, Kolber Z, Berry JA, Falkowski PG (1996) Cyclic electron flow around photosystem II *in vivo*. Photosynth Res **48:** 395–410

Quick WP, Stitt M (1989) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim Biophys Acta **977:** 287–296

Raison JK, Roberts JKM, Berry JA (1982) Correlation between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant *Nerium oleander* to growth temperature. Biochim Biophys Acta **688:** 218–228

Rees D, Noctor GD, Horton P (1990) The effect of high-energy-state excitation quenching on maximum and dark level fluorescence yield. Photosynth Res **25:** 199–211

Routaboul J-M, Fischer SF, Browse J (2000) Trienoic fatty acids are required to maintain chloroplast function at low temperature. Plant Physiol **124:** 1697–1705

Russell AW, Critchley C, Robinson SA, Franklin LA, Seaton GGR, Chow WS, Anderson JM, Osmond CB (1995) Photosystem II regulation and dynamics of the chloroplast D1 protein in Arabidopsis leaves during photosynthesis and photoinhibition. Plant Physiol **107:** 943–952

Sane PV, Ivanov AG, Sveshnikov D, Huner NPA, Öquist G (2002) A transient exchange of the photosystem II reaction center protein D1:1 with D1:2 during low temperature stress of *Synechococcus* sp. PCC 7942 in the light lowers the redox potential of Q_B. J Biol Chem **277:** 32739–32745

Sane PV, Rutherford AW (1986) Thermoluminescence from photosynthetic membranes. *In* Govindjee, J Amesz, DC Fork, eds, Light Emission by Plants and Bacteria. Academic Press, Orlando, FL, pp 329–360

Savitch LV, Barker-Åström J, Ivanov AG, Hurry V, Öquist G, Huner NPA, Gardeström P (2001) Cold acclimation of *Arabidopsis thaliana* results in incomplete recovery of photosynthetic capacity, associated with an increased reduction of the chloroplast stroma. Planta **214:** 295–303

Schatz GH, Brock H, Holzwarth AR (1988) Kinetic and energetic model for the primary processes in photosystem II. Biophys J **54:** 397–405

Stitt M, Hurry V (2002) A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis. Curr Opin Plant Biol **5:** 199–206

Strand Å, Foyer CH, Gustafsson P, Gardeström P, Hurry V (2003) Altering flux through the sucrose biosynthesis pathway in transgenic *Arabidopsis thaliana* modifies photosynthetic acclimation at low temperatures and the development of freezing tolerance. Plant Cell Environ **26:** 523–535

Strand Å, Hurry V, Gustafsson P, Gardeström P (1997) Development of *Arabidopsis thaliana* leaves at low temperature releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. Plant J **12:** 605–614

Strand Å, Hurry V, Henkes S, Huner N, Gustafsson P, Gardeström P, Stitt M (1999) Acclimation of Arabidopsis leaves developing at low temperatures: Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin cycle and in the sucrose/biosynthesis pathway. Plant Physiol **119:** 1387–1398

Vass I, Govindjee (1996) Thermoluminescence from the photosynthetic apparatus. Photosynth Res **48:** 117–126

Vass I, Styring S, Hundal T, Koivuniemi A, Aro E-M, Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced Q_A species promote chlorophyll triplet formation. Proc Natl Acad Sci USA **89:** 1408–1412

Vavilin DV, Vermass WFJ (2000) Mutations in the CD-loop region of the D2 protein in *Synechocystis* sp. PCC 6803 modify charge recombination pathways in photosystem II in vivo. Biochemistry **39:** 14831–14838

Weis E, Berry JA (1987) Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta **894:** 198–208