FEBS Letters 580 (2006) 2797–2802

Acclimation to temperature and irradiance modulates PSII charge recombination

A.G. Ivanov[a,1], P.V. Sane[a,1], M. Krol[a], G.R. Gray[b], A. Balseris[a], L.V. Savitch[c], G. Öquist[d], N.P.A. Hüner[a,*]

[a] *Department of Biology and The Biotron, University of Western Ontario, London, Canada N6A 5B7*
[b] *Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada S7N 5A8*
[c] *Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada K1A OC6*
[d] *Umeå Plant Science Center, Department of Plant Physiology, University of Umeå, Umeå SE-90187, Sweden*

Received 14 March 2006; revised 7 April 2006; accepted 10 April 2006

Available online 21 April 2006

Edited by Richard Cogdell

Abstract Acclimation of wild type and the *chlorina F2* mutant of barley to either high light or low temperature results in a 2- to 3-fold increase in non-photochemical quenching which occurred independently of either energy-dependent quenching (qE), xanthophyll cycle-mediated antenna quenching or state transitions. Results of in vivo thermoluminescence measurements used to address this conundrum indicated that excitation pressure regulates the temperature gap for $S_2Q_B^-$ and $S_2Q_A^-$ charge recombinations within photosystem II reaction centers. This is discussed in terms of photoprotection through non-radiative charge recombination.

Keywords: Acclimation; Charge recombination; Irradiance; PSII; Temperature

1. Introduction

Changes in either irradiance or temperature exacerbate the potential imbalances in energy budget due to imbalances between the rate at which photosynthetic electrons are generated through photochemistry versus the rate at which redox potential energy is utilized through reductive metabolism [1–3]. Thus, either high light or low temperature can result in comparable excitation pressure estimated as 1 − qP [1–3].

Although PSII is a major component regulating photosynthetic linear electron transport, it is also extremely susceptible to photoinhibition and photodamage upon exposure to excess excitation [4–6]. Non-photochemical quenching (NPQ) through xanthophyll-dependent antenna quenching [7–10] and state transitions [11,12] represent major advances in our understanding of photoprotection of PSII. However, several reports have indicated that significant levels of NPQ can not be accounted for by xanthophyll cycle-dependent, antenna quenching [7,13–17]. Chl *a* fluorescence quenching analyses [4,14–19] as well as thermoluminescence measurements [15,20,21] indicate that reaction center quenching may contribute to photoprotection of PSII [4]. We combined thermoluminescence, a very sensitive spectroscopic technique to probe charge recombination events in PSII reaction centers in vivo [22–26], with Chl *a* fluorescence induction and P700 measurements to test the hypothesis that excitation pressure modulates PSII charge recombination events in response to growth and acclimation to high light and low temperature. The results are discussed in terms of photoprotection through a non-radiative pathway for quenching within PSII reaction centers.

2. Materials and methods

2.1. Growth conditions

WT and the *F*2 mutant of barley (*Hordeum vulgare* L.) and winter rye (*Secale cereale* L.) were grown in controlled environment chambers (Conviron, Winnipeg, MB, Canada) at four different temperature/irradiance conditions (°C/μmol photons m^{-2} s^{-1}): 20/250, 20/800, 5/50, 5/250 and a relative humidity of 50%. Fully expanded second and third leaves harvested 2 h into the photoperiod were used in all experiments.

2.2. Pigment analyses

Pigments were extracted, separated and quantified by high-performance liquid chromatography as described in detail previously [27]. The epoxidation state (EPS) of the xanthophyll cycle pigments was calculated as: EPS = (V + 0.5A)/(V + A + Z). V, violaxanthin; A, antheraxanthin; Z, zeaxanthin.

2.3. SDS–PAGE and immunoblotting

Thylakoid membranes for SDS–PAGE were isolated as described in detail previously [28]. Solubilized samples containing equal amounts of protein (20 μg $lane^{-1}$) were separated on a 15% (w/v) linear polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes. Immunoblots were performed with specific antibodies raised against D1 protein (PsbA), Lhcb1, Lhcb5 and PsbS (1:5000 dilutions).

2.4. Modulated chlorophyll fluorescence

Modulated chlorophyll *a* fluorescence of dark adapted leaves was measured under ambient O_2 and CO_2 with a PAM 101 chlorophyll fluorescence measuring system (Heinz Walz GmbH, Effeltrich, Germany). NPQ and 1 − qP were calculated as described in [3]. All fluorescence parameters were measured at the corresponding growth temperature and growth irradiance during steady-state photosynthesis.

*Corresponding author. Fax: +1 519 661 3935.
E-mail address: nhuner@uwo.ca (N.P.A. Hüner).

[1] These authors contributed equally to this research.

Abbreviations: EPS, epoxidation state; F_v/F_m, maximum photochemical efficiency of PSII in the dark adapted state; NPQ, non-photochemical quenching; qP, photochemical quenching parameter; ST, capacity for state transition calculated as $[(F'_I - F_I) - (F'_{II} - F_{II})]/(F'_I - F_I)$; T_M, thermoluminescence peak temperature

0014-5793/$32.00
doi:10.1016/j.febslet.2006.04.018

State 1 and state 2 transitions were estimated at the corresponding growth temperature/irradiance conditions as described by Lunde et al. [11], using PAM-101 chlorophyll fluorescence measuring system (Heinz Walz GmbH, Effeltrich, Germany) equipped with a blue light source consisting of a lamp with a Corning 4-96 filter and a far red light (FR) was provided by a FL-101 light source; (λ_{max} = 715 nm, 10 W m^{-2}, Schott filter RG 715). The relative change in state transition was calculated $[(F'_I - F_I) - (F'_{II} - F_{II})]/(F'_I - F_I)$, where F_I and F_{II} designate fluorescence in the presence of PSI light (far-red) in state 1 and state 2, respectively, while F'_I and F'_{II} designate fluorescence in the absence of PSI light in state 1 and state 2, respectively [11].

2.5. Thermoluminescence

Thermoluminescence (TL) measurements of intact barley and rye leaves were performed on a custom-designed, personal-computer-based TL data acquisition and analysis system as described earlier [20,21]. A xenon-discharge flash lamp (XST103, Heinz Walz GmbH, Effeltrich, Germany) was used to expose the samples to a single turnover flash (1.5 μs peak width at 50% of maximum). Dark adapted leaves were cooled to 0 °C prior to exposing the samples to the flashes. For $S^2Q_A^-$ recombination studies, leaves were vacuum infiltrated with DCMU (20 μM) in darkness before the flash illumination. Experiments were performed at a heating rate of 0.6 °C s^{-1}.

2.6. e/P700

The relative intersystem electron pool sice (e/P700) was measured by the photooxidation of P_{700} estimated as the absorbance change at 820 nm ($\Delta A_{820}/A_{820}$) at the respective growth temperatures in winter rye as described in detail previously [20,29].

3. Results and discussion

We confirmed [30,31] that the *F*2 mutant exhibited minimal levels of Lhcb1 but comparable levels of the PSII reaction center polypeptide, D1, and the core antenna polypeptide, Lhcb5 compared to WT barley regardless of the growth condition (20/250, 20/800, 5/50, 5/250) (Fig. 1A). Neither WT barley nor the *F*2 mutant exhibited any signs of photoinhibition during steady-state growth as indicated by comparable maximal PSII photochemical efficiency (F_v/F_m = 0.787 ± 0.015) in leaves in their dark adapted state under all regimes tested. Similar results have been reported for rye and wheat [32]. Nevertheless, excitation pressure measured as 1 − qP [3] in the WT acclimated to either 5/250 or 20/800 was 2- to 3-fold higher than that of barley acclimated to either 5/50 or 20/250 (Table 1). Similar results for the effects of growth regime on 1 − qP were observed for the *F*2 mutant (Table 2) and have been published for winter rye [32].

Concomitantly, NPQ increased 2- to 3-fold in WT barley plants acclimated to high excitation pressure (20/800 or 5/250) compared to plants grown at low excitation pressure (20/250 or 5/50), respectively. However, the changes in NPQ were associated with only minimal changes in the EPS of the xanthophyll cycle (Table 1). Similar trends for the effects of steady-state growth regime on NPQ (Table 2) were observed in the *F*2 mutant and have been reported for winter rye [32]. Thus, although changes in NPQ are correlated with alterations in excitation pressure during steady-state growth of WT bar-

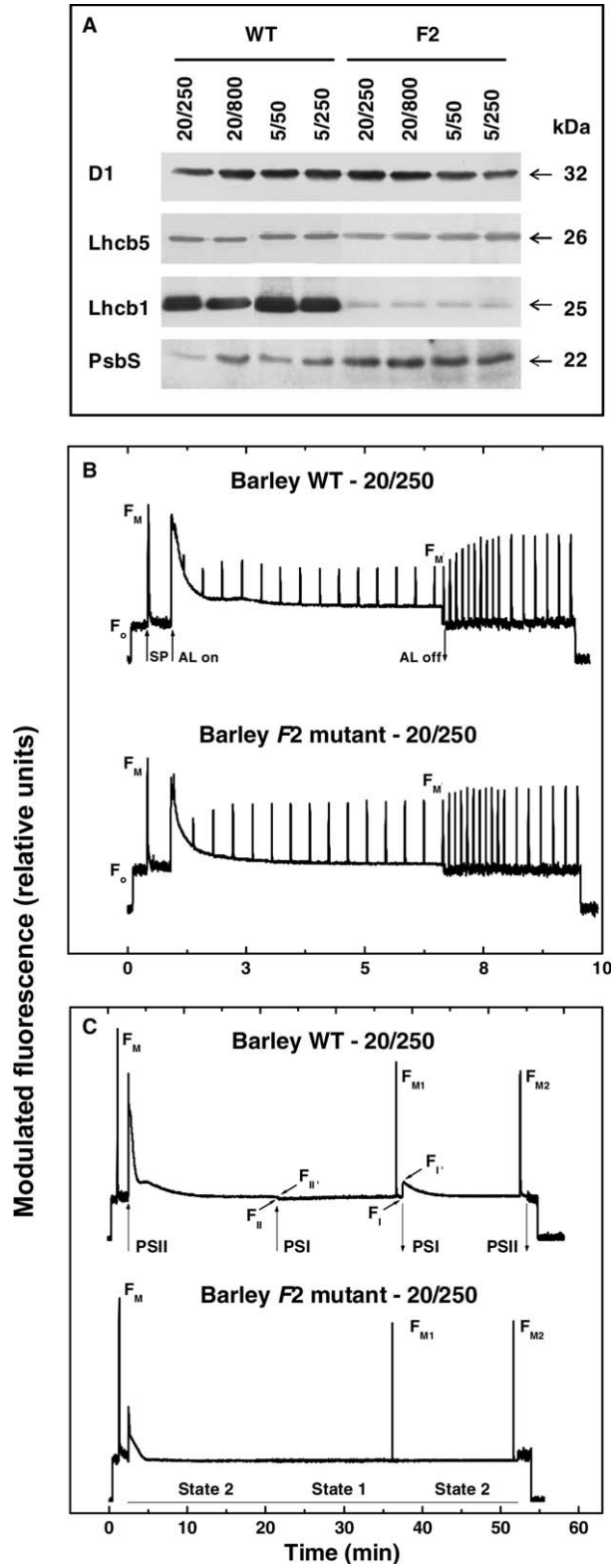

Fig. 1. (A) Immunoblot analysis of WT and *F*2 thylakoid membrane proteins isolated from plants grown under different temperature (°C)/irradiance (μmol m^{-2} s^{-1}) regimes. (B) Chl *a* fluorescence induction curves for WT and the *F*2 mutant of barley. Up arrows indicate actinic white light (250 μmol photons m^{-2} s^{-1}) on. Down arrows indicate actinic light off. (C) Typical fluorescence traces of state 1–state 2 transitions in WT and *F*2 mutant of barley grown at 20 °C and an irradiance of 250 μmol photons m^{-2} s^{-1}. Dark adapted leaves were exposed to either blue light favoring PS II or far-red light favoring PS I as described in detail by Lunde et al. [11]. Up arrows indicate lights on and down arrows indicate lights off. The scale at the bottom of indicates time in state 2 and state 1.

Table 1
Effect of growth irradiance and growth temperature on photosynthetic parameters and the T_M of $S_2Q_A^-$ and $S_2Q_B^-$ thermoluminescence glow peaks of WT barley

Growth regime (°C/μmol m^{-1} s^{-1})	1 − qP	EPS	NPQ	$T_M(S_2Q_B^-)$ (°C)	$T_M(S_2Q_A^-)$ (°C)	ΔT_M (°C)
20/250	0.287 ± 0.024	0.90 ± 0.03	1.21 ± 0.05	41.1 ± 0.3	20.9 ± 0.1	20.1 ± 0.2
20/800	0.619 ± 0.016	0.75 ± 0.05	2.42 ± 0.08	35.7 ± 0.6	23.8 ± 0.2	11.8 ± 0.4
5/50	0.100 ± 0.026	0.93 ± 0.03	0.72 ± 0.18	39.3 ± 1.3	22.8 ± 0.1	16.5 ± 0.7
5/250	0.465 ± 0.055	1.00 ± 0.03	2.32 ± 0.09	35.2 ± 0.2	28.9 ± 0.2	6.3 ± 0.2

All measurements were performed at the growth temperature and growth irradiance. All data are the means of 3–5 experiments ± S.E. with at least three replicate measurements per experiment. $\Delta T_M = T_M(S_2Q_B^-) - T_M(S_2Q_A^-)$.

Table 2
Effect of growth irradiance and growth temperature on photosynthetic parameters (1 − qP, NPQ), EPS of the xanthophylls cycle pigments and the characteristic temperatures (T_M) of $S_2Q_A^-$ and $S_2Q_B^-$ thermoluminescence glow peaks of the barley *F*2 mutant

Growth regime (°C/μmol m^{-1} s^{-1})	1 − qP	EPS	NPQ	$T_M(S_2Q_A^-)$ (°C)	$T_M(S_2Q_A^-)$ (°C)	ΔT_M (°C)
20/250	0.132 ± 0.003	0.93 ± 0.02	0.45 ± 0.02	40.4 ± 0.1	25.3 ± 1.4	15.1 ± 0.3
20/800	0.527 ± 0.045	0.83 ± 0.02	1.11 ± 0.05	37.2 ± 0.2	30.2 ± 2.4	7.0 ± 0.3
5/50	0.106 ± 0.002	1.00 ± 0.02	0.65 ± 0.07	41.9 ± 0.8	26.5 ± 1.4	15.4 ± 1.2
5/250	0.425 ± 0.047	0.95 ± 0.04	0.81 ± 0.07	34.8 ± 1.0	29.7 ± 0.7	5.1 ± 0.5

All measurements were performed at the growth temperature and growth irradiance. All data are the means of 3–5 experiments ± S.E. with at least three replicate measurements per experiment. $\Delta T_M = T_M(S_2Q_B^-) - T_M(S_2Q_A^-)$.

ley, the *F*2 mutant and winter rye, the changes in NPQ occur independently of changes in xanthophyll-cycle activity.

There is a consensus that the major component of NPQ is the ΔpH-dependent quenching (qE) [8,33]. The initial, fast phase of the recovery of Chl *a* fluorescence flash yields after the actinic light has been turned off, can be used to estimate the extent of qE [8,33]. As shown in Fig. 1B, the *F*2 mutant exhibited minimal capacity for qE compared to WT barley. State 1–state 2 transitions represent a short-term mechanism to balance the energy distribution between PSII and PSI [11,12]. Fig. 1C illustrates that WT barley exhibited normal capacity to undergo state transitions when grown at low excitation pressure at 20/250 ($ST = [(F'_I - F_I) - (F'_{II} - F_{II})]/(F'_I - F_I) = 0.744 \pm 0.090$). Although WT barley grown at low excitation pressure at 5/50 also exhibited a similar capacity for state transitions (ST = 0.629 ± 0.048) as WT grown at 20/250, growth of WT at high excitation pressure at either 20/800 (ST = 0.445 ± 0.031) or 5/250 (ST = 0.327 ± 0.063) inhibited ST by about 45%. In contrast to WT, we were unable to detect state transitions in the *F*2 mutant grown at either 20/250 (Fig. 1C) or at any of the growth conditions tested (data not shown). Thus, the modulation of NPQ in WT barley (Table 1) and the *F*2 mutant (Table 2) in response to changes in excitation pressure can not be accounted for by changes in either xanthophyll-cycle activity or qE. Although Lhcb5 (CP26) has been shown to be a requirement for state transitions in *Chlamydomonas reinhardtii* [34], enhanced capacity for state transitions can not account the modulation of NPQ by excitation pressure in either the WT or the *F*2 mutant of barley (Fig. 1C; Table 2).

Chl *a* fluorescence quenching analyses [4,14–19] as well as thermoluminescence measurements [15,20,21] indicate that reaction center quenching may contribute to NPQ. Fig. 2 illustrates typical thermoluminescence glow curves for $S_2Q_B^-$ and $S_2Q_A^-$ recombinations in WT barley. WT barley grown at high excitation pressure (Fig. 2C and D) exhibited a narrowing of the temperature gap (ΔT_M) between $S_2Q_B^-$ (−DCMU) and $S_2Q_A^-$ (+DCMU) recombinations compared to WT barley grown at low excitation pressure (Fig. 2A and B). The T_M for $S_2Q_B^-$ recombination was down-shifted by 4–5 °C whereas the T_M for the $S_2Q_A^-$ recombination was up-shifted by 3–5 °C in WT barley grown at high excitation pressure (20/800 or 5/250) relative to WT grown at low excitation pressure (20/250 or 5/50) (Table 1). Excitation pressure had similar effects on the T_M of the $S_2Q_B^-$ and $S_2Q_A^-$ recombinations in the *F*2 mutant. The ΔT_M for the *F*2 mutant grown at either 20/250 or 5/50 was 15.1 ± 0.3 and 15.4 ± 1.2 °C, respectively, whereas ΔT_M for growth at either 20/800 or 5/250 was 7.0 ± 0.3 and 5.1 ± 0.5 °C, respectively. Thus, high excitation pressure appears to cause a similar 8–10 °C narrowing of the ΔT_M for $S_2Q_A^-$ and $S_2Q_B^-$ recombinations in WT and the *F*2 mutant of barley.

PsbS is the PSII subunit assumed to be essential for NPQ via the xanthophyll cycle [9,10,35]. Although the content of PsbS appeared to be correlated with excitation pressure in WT barley (Fig. 1A), excitation pressure had no effect on PsbS levels in the *F*2 mutant (Fig. 1A). To eliminate the possibility that changes in the levels of PsbS may affect PSII charge recombination and ΔT_M, we also compared $S_2Q_A^-/S_2Q_B^-$ recombinations in the *npq4-1* mutant of *Arabidopsis thaliana*, which lacks PsbS and exhibits minimal NPQ [9,10], with those in WT *A. thaliana*. We observed a 5.7 °C down-shift in the T_M for $S_2Q_A^-$ recombinations from 39.9 ± 0.6 °C in the WT to 34.2 ± 0.7 °C in the *npq4-1* mutant and a concomitant 4.9 °C down-shift in the T_M for $S_2Q_A^-$ recombinations from 22.5 ± 0.6 °C in the WT to 17.6 ± 0.6 °C in *npq4-1* mutant. Although charge recombination is sensitive to PsbS, the presence or absence of PsbS did not affect the ΔT_M for $S_2Q_A^-$ / $S_2Q_B^-$ recombinations in the *npq4-1* mutant (ΔT_M = 16.6 °C) compared to WT *A. thaliana* (ΔT_M = 17.4 °C).

The pooled results for WT barley, the *F*2 mutant and winter rye grown at either 20/250, 20/800, 5/50 or 5/250 illustrated in Fig. 2E indicate that the ΔT_M for $S_2Q_A^-$ and $S_2Q_B^-$ recombinations decreased in a linear fashion from about 25 °C at low 1 − qP to about 6 °C at high values of 1 − qP. A decrease in ΔT_M is consistent with a decrease in the free energy gap for electron transfer between Q_A and Q_B [22–24,36] which creates a predisposition for the accumulation of Q_A^- and non-radiative

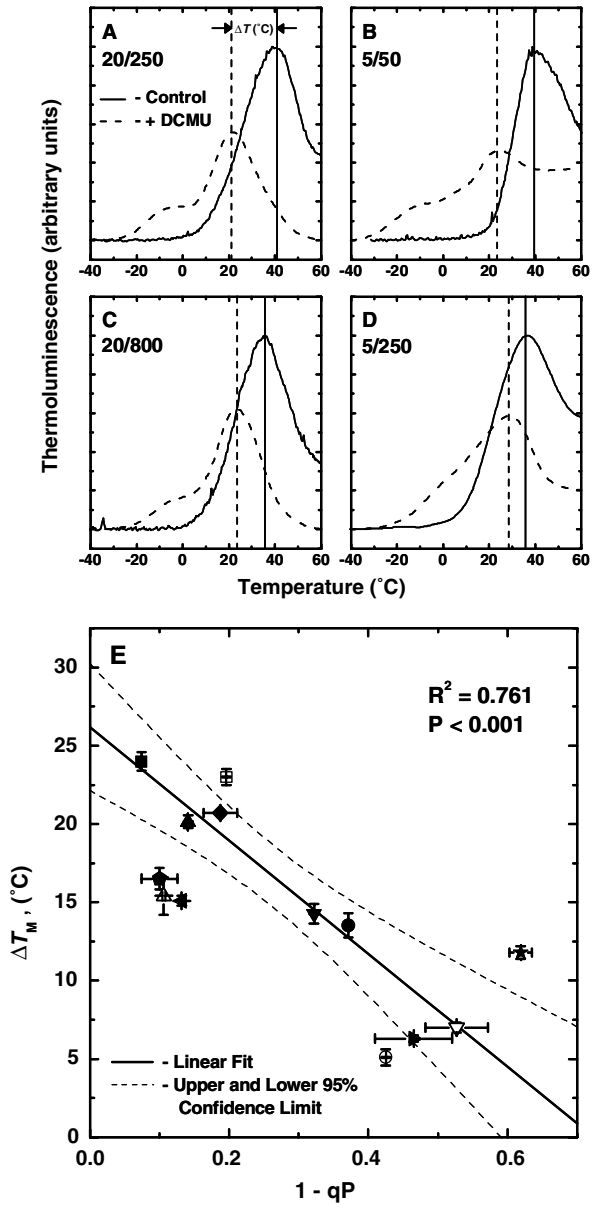

Fig. 2. Thermoluminescence glow curves of $S_2Q_B^-$ (solid curves) and $S_2Q_A^-$ (dashed curves) charge recombinations in WT barley plants grown under low (A, B) and high (C, D) excitation pressure conditions. Experimental curves represent averages of 5–9 scans. Vertical broken and solid lines represent T_M for $S_2Q_A^-$ and $S_2Q_B^-$ recombinations, respectively, and the ΔT_M represents the temperature gap between the $S_2Q_A^-$ and $S_2Q_B^-$ recombinations. (E) Correlation between the reduction state of Q_A measured as $1 - qP$ and the temperature gap (ΔT_M) between the characteristic peak temperatures (T_M) of $S_2Q_B^-$ and $S_2Q_A^-$ charge recombinations in WT and *F*2 mutant barley and rye plants grown at either 20/50, 20/250, 20/800, 5/50 or 5/250. All measurements of $1 - qP$ were performed at the corresponding growth temperature and irradiance. All values represent means ± S.E. from 3 to 5 independent measurements. The *P*-value refers to the linear regression. (♦) WT-barley-20/250; (★) WT-barley-20/800; (⬟) WT barley-5/50; (▶) WT barley-5/250; (◀) *F*2 barley-20/250; (▽) *F*2 barley-20/800; (△) *F*2 barley-5/50; (○) *F*2 barley-5/250; (□) Rye-20/250; (▼) Rye-20/800; (▲) Rye-5/50; (●) Rye-5/250; (■) Rye-20/50.

PSII charge recombination. Although modulation of the ΔT_M for $S_2Q_A^-$ and $S_2Q_B^-$ recombinations by low growth temperature have been reported for *Synechococcus* sp PCC 7942 [21], *A. thaliana* [15] as well as for pine exposed to natural over-wintering conditions [20], we show for the first time that excitation pressure rather than low temperature per se regulates ΔT_M for $S_2Q_A^-$ and $S_2Q_B^-$ recombinations.

Fig. 3A illustrates that an increase in $1 - qP$ is positively correlated with an increase in the intersystem electron pool size (e^-/P700). We conclude that steady-state growth at either 20/800 or 5/250 results in comparable excitation pressure ($1 - qP$) because both growth regimes induce comparable metabolic limitations on the acceptor side of PSI, which cause an increased reduction state of the PQ pool. This, in turn, induces a decrease in the ΔT_M (Fig. 3B). Our results indicate that PSII reaction centers are functionally altered in response to modulation of the redox state of the intersystem electron transport

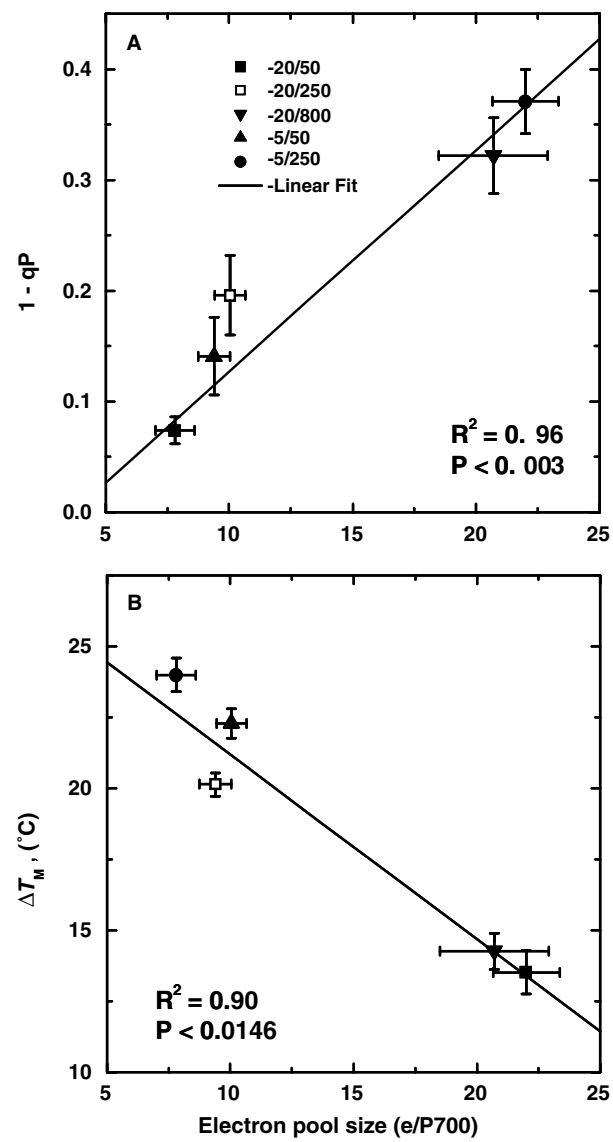

Fig. 3. (A) Relationship between excitation pressure ($1 - qP$) and intersystem electron pool size (e^-/P700) in winter rye. All plants were grown at either 20/50, 20/250, 20/800, 5/50 or 5/250. Excitation pressure ($1 - qP$) was calculated as described in [3], e^-/P700 was calculated according to [29,38] and all measurements were performed at the corresponding growth temperature and irradiance. (B) Relationship between ΔT_M and e^-/P700. All data represent the means ± S.E. from 3 to 5 independent experiments.

chain such that the free energy gap between $S_2Q_A^-$ and $S_2Q_B^-$ is decreased favoring non-radiative PSII charge recombination when the PQ pool is reduced during steady-state growth. We have reported that such decreases in the ΔT_M are associated with a 30–40% decrease in total thermoluminescence yield [15,21] which is consistent with a dark decay pathway for these charge recombinations events [37] and photoprotection through non-radiative reaction center quenching. Thus, our results support the suggestion [4] that photoprotection of PSII through reaction center quenching complements photoprotection through antenna quenching.

Acknowledgements: Supported by the Natural Sciences and Engineering Research Council of Canada and the Swedish Research Council. We thank K. Niyogi for providing us with the *npq4-1* mutant of *Arabidopsis thaliana*.

References

[1] Falkowski, P.G. and Chen, Y.-B. (2003) Photoacclimation of light harvesting systems in eukaryotic algae in: Advances in Photosynthesis and Respiration (Green, B.R. and Parson, W.W., Eds.), Light Harvesting Systems in Photosynthesis, vol. 13, pp. 423–447, Kluwer Academic Publishers, Dordrecht.

[2] Anderson, J.M., Chow, W.S. and Park, Y.-I. (1995) The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. Photosynth. Res. 46, 129–139.

[3] Huner, N.P.A., Öquist, G. and Sarhan, F. (1998) Energy balance and acclimation to light and cold. Trends Plant Sci. 3, 224–230.

[4] Krause, G.H. and Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349.

[5] Melis, A. (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? Trends Plant Sci. 4, 130–135.

[6] Karpinski, S., Reynolds, H., Karpinska, B., Wingsle, G., Creissen, G. and Mullineaux, P. (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science 284, 654–657.

[7] Demmig-Adams, B., Adams, W.W., Ebbert, V. and Logan, B.A. (1999) Ecophysiology of the xanthophyll cycle in: Advances in Photosynthesis (Frank, H.A., Young, A.J., Britton, G. and Cogdell, R.J., Eds.), The Photochemistry of Carotenoids, vol. 8, pp. 245–269, Kluwer Academic Publishers, Dordrecht.

[8] Horton, P., Ruban, A.V. and Young, A.J. (1999) Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll cycle in: Advances in Photosynthesis (Frank, H.A., Young, A.J., Britton, G. and Cogdell, R.J., Eds.), The Photochemistry of Carotenoids, vol. 8, pp. 271–291, Kluwer Academic Publishers, Dordrecht.

[9] Niyogi, K.K., Li, X.-P., Rosenberg, V. and Jung, H.-S. (2005) Is PsbS the site of non-photochemical quenching in photosynthesis? J. Exp. Bot. 56, 375–382.

[10] Holt, N.E., Zigmantas, D., Valkunas, L., Li, X.-P., Niyogi, K.K. and Fleming, G.R. (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. Science 307, 433–436.

[11] Lunde, C., Jensen, P.E., Haldrup, A., Knoetzel, J. and Scheller, H.V. (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408, 613–615.

[12] Depege, N., Bellafiore, S. and Rochaix, J.-D. (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. Science 299, 1572–1575.

[13] Dall'Osto, L., Caffarri, S. and Bassi, R. (2005) A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. Plant Cell 17, 1217–1232.

[14] Bukhov, N.G., Heber, U., Wiese, C. and Shuvalov, V.A. (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.

[15] Sane, P.V., Ivanov, A.G., Hurry, V., Huner, N.P.A. and Öquist, G. (2003) 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.

[16] Kopecky, J., Azarkovich, M., Pfundel, E., Shuvalov, V.A. and Heber, U. (2005) Thermal dissipation of light is regulated differently and by different mechanisms in lichens and higher plants. Plant Biol. 7, 156–167.

[17] Matsubara, S. and Chow, W.S. (2004) Populations of photoinactivated photosystem II reaction centers characterized by chlorophyll a fluorescence lifetime in vivo. Proc. Natl. Acad. Sci. USA 101, 18234–18239.

[18] Finazzi, G., Johnson, G.N., Dall'Osto, L., Joliot, P., Wollman, F.-A. and Bassi, R. (2004) A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. Proc. Natl. Acad. Sci. USA 101, 12375–12380.

[19] Weis, E. and Berry, J. (1987) Quantum efficiency of photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 894, 198–208.

[20] Ivanov, A.G., Sane, P.V., Zeinalov, Y., Simidjiev, I., Huner, N.P.A. and Öquist, G. (2002) Seasonal responses of photosynthetic electron transport in Scots pine (*Pinus sylvestris* L.) studies by thermoluminescence. Planta 215, 457–465.

[21] Sane, P.V., Ivanov, A.G., Sveshnikov, D., Huner, N.P.A. and 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.

[22] Sane, P.V. and Rutherford, W.A. (1986) Thermoluminescence from photosynthetic membranes in: Light Emission by Plants and Bacteria (Govindjee, Amesz, J. and Fork, D.J., Eds.), pp. 329–360, Academic Press, Orlando.

[23] Inoue, Y. (1996) Photosynthetic luminescence as a simple probe of photosystem II electron transport in: Advances in Photosynthesis (Amesz, J. and Hoff, A.J., Eds.), Biophysical Techniques in Photosynthesis, vol. 3, pp. 93–107, Kluwer Academic Publishers, Dordrecht.

[24] Ducruet, J.-M. (2003) Chlorophyll thermoluminescence of leaf discs: simple instruments and progress in signal interpretation open the way to new ecophysiological indicators. J. Exp. Bot. 54, 2419–2430.

[25] Rappaport, F., Cuni, A., Xiong, L., Sayre, R. and Lavergne, J. (2005) Charge recombination and thermoluminescence in photosystem II. Biophys. J. 88, 1948–1958.

[26] Vass, I. and Govindjee (1996) Thermoluminescence from the photosynthetic apparatus. Photosynth. Res. 48, 117–126.

[27] Ivanov, A.G., Krol, M., Maxwell, D. and Huner, N.P.A. (1995) Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle. FEBS Lett. 371, 61–64.

[28] Krol, M., Ivanov, A.G., Jansson, S., Kloppstech, K. and Huner, N.P.A. (1999) Greening under high light or cold temperature affects the level of xanthophyll-cycle pigments, early light-inducible proteins, and light-harvesting polypeptides in wild-type barley and the *chlorina f2* mutant. Plant Physiol. 120, 193–204.

[29] Asada, K., Heber, U. and Schreiber, U. (1992) Pool size of electrons that can be donated to $P700^+$ as determined in intact leaves: donation to $P700^+$ from stromal components via the intersystem chain. Plant Cell Physiol. 33, 927–932.

[30] Ghirardi, M.L., McCauley, S.W. and Melis, A. (1986) Photochemical apparatus organization in the thylakoid membrane of *Hordeum vulgare* wild-type and chlorophyll *b*-less *chlorina-f2* mutant. Biochim. Biophys. Acta 934, 151–155.

[31] Harrison, M.A., Nemson, J.A. and Melis, A. (1993) Assembly and composition of the chlorophyll *a–b* light harvesting complex of barley (*Hordeum vulgare* L.): immunochemical analysis of chlorophyll *b*-less and chlorophyll *b*-deficient mutants. Photosynth. Res. 38, 141–151.

[32] Gray, G.R., Savitch, L.V., Ivanov, A.G. and Huner, N.P.A. (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.

[33] Horton, P. and Ruban, A. (2005) Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. J. Exp. Bot. 56, 365–373.

[34] Takahashi, H., Iwai, M., Takahashi, Y. and Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in *Chlamydomonas reinhardtii*.

[35] Crouchman, S., Ruban, A. and Horton, P. (2005) PsbS enhances nonphotochemical fluorescence quenching in the absence of zeaxanthin. FEBS Lett. 580, 2053–2058.

[36] DeVault, D. and Govindjee (1990) Photosynthetic glow peaks and their relationship with free energy changes. Photosynth. Res. 24, 175–181.

[37] Vavilin, D.V. and Vermaas, W.F.J. (2000) Mutations in the CD-loop region of the D2 protein in *Synechocystis* PCC 6803 modify charge recombination pathways in photosystem II in vivo. Biochemistry 39, 14831–14838.

[38] Gray, G.R., Ivanov, A.G., Krol, M. and Huner, N.P.A. (1998) Adjustment of thylakoid plastoquinone content and electron donor pool size in response to growth temperature and growth irradiance in winter rye (*Secale cereale* L.). Photosynth. Res. 56, 209–221.