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

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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 \( S_{2}Q_{\Delta}^- \) recombination was shifted to higher temperatures, whereas the characteristic temperature of the \( S_{2}Q_{\Delta}^- \) recombination was shifted to lower temperatures in cold-acclimated plants. These shifts in recombination temperatures indicate higher activation energy for the \( S_{2}Q_{\Delta}^- \) redox pair and lower activation energy for the \( S_{2}Q_{\Delta}^- \) redox pair. This results in an increase in the free-energy gap between \( P_{680}Q_{\Delta}^- \) and \( P_{680}Pheo^- \) 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 \( P_{680}Q_{\Delta}^- \) 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 (\( Q_{\Delta} \)) 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 \( 1 - q_{p} = [ (Q_{\Delta})_{\text{red}} / (Q_{\Delta})_{\text{ox}} ] \) 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

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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 photo-inhibition 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 redox recombination events between the acceptor and donor sides of PSII during acclimation of Arabidopsis plants to low temperature.

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 (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-dimethyurea (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 $S_{2}Q_{B}$ and $S_{2}Q_{B}^{–}$ 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 QA and the secondary electron-accepting quinone in PSII (Qb) and concomitant conversion of $S_{2}Q_{B}^{–}$ to $S_{2}Q_{A}^{–}$ (Inoue, 1996), the data presented above clearly show that the peak appearing around 38°C in the absence of DCMU (Fig. 2A) originates from $S_{2}Q_{B}^{–}$ recombination, whereas the appearance of DCMU-resistant peak around −18°C (Fig. 2C) is ascribed to the $S_{2}Q_{A}^{–}$ recombination (Inoue, 1996; Vass and Govindjee, 1996). The TL peaks with characteristic $T_{M}$ above 50°C (Fig. 2, A–D)
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). In contrast, the shift in the S2QA- and S2QB- 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 S2QB peak gradually decreased, the T_M of S2QA shifted to higher temperatures. Thus, the initial gap of 55°C between S2QA and S2QB 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 re-absorption 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 states in centers in which Q_A is poorly connected to Q_B and the plastocyanin 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.
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

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**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_M$</th>
<th>A (%)</th>
<th>$T_M$</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td></td>
<td>°C</td>
<td></td>
</tr>
<tr>
<td>Non-hardened</td>
<td>-12.0 ± 0.06</td>
<td>5.1 ± 0.1</td>
<td>-17.6 ± 0.08</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>37.7 ± 0.03</td>
<td>67.8 ± 0.1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>53.9 ± 0.06</td>
<td>27.1 ± 0.1</td>
<td>58.7 ± 0.08</td>
<td>92.1 ± 0.2</td>
</tr>
<tr>
<td>Cold acclimated</td>
<td>0.3 ± 0.1</td>
<td>9.1 ± 0.1</td>
<td>-8.1 ± 0.02</td>
<td>25.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>28.3 ± 0.02</td>
<td>45.6 ± 0.1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>48.6 ± 0.06</td>
<td>45.3 ± 0.2</td>
<td>55.3 ± 0.07</td>
<td>74.5 ± 0.2</td>
</tr>
</tbody>
</table>

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**Figure 3. A, Time course of the characteristic $T_M$ of $S_2Q_A^-$ and $S_2Q_B^-$ 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.**
may have changed. In addition, the possibility that the redox characteristics of the oxidizing species (S2 and S3 in this case) participating in the recombination reactions may also have changed cannot be ruled out.

However, if there had been a change in the redox characteristics of the S2/S3 states, the change should have been in the same direction. Our observations indicate that the S2QA+/H1002 peak was shifted toward higher temperature, whereas the S2QB+/H1002 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 QA and/or QB.

The high-temperature shift in the $T_M$ of S2QA−, corresponding to increased activation energy of QA/QA−, would increase the free energy gap between P680−QA− and P680−Pheo−. This could cause stabilization of S2QA− 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 QA in cold-acclimated Arabidopsis could also result from a change in the redox potential of QB. Lowering the redox potential of QB will narrow the gap between the redox potentials between QA and QB even further and will decrease the probability for forward electron transfer between the two quinone acceptors by shifting the redox equilibrium between QA− QB and QAQB− toward QA− QB (Fig. 6; Minagawa et al., 1999). The retention of electrons preferentially on QA through a modification of the redox potentials of QA and QB in opposite directions may cause the reoxidation of QA− by forward and/or back reaction to

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**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 ($t_{1/2}$) and amplitude (A). Mean values ± se were calculated from eight to 10 recordings.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cold Acclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{fast}$ (μs)</td>
<td>201 ± 19</td>
<td>340 ± 67</td>
</tr>
<tr>
<td>$t_{int}$ (μs)</td>
<td>1,350 ± 56</td>
<td>4,990 ± 363</td>
</tr>
<tr>
<td>$A_{fast}$ (%)</td>
<td>43.3</td>
<td>42.0</td>
</tr>
<tr>
<td>$A_{int}$ (%)</td>
<td>23.9</td>
<td>36.7</td>
</tr>
<tr>
<td>$X^2$</td>
<td>0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>
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 QA 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 QA at the given growth temperature (Huner et al., 1998). Moreover, our recent data also indicated an increased proportion of QA− (measured as 1-qP) in cold-acclimated Arabidopsis (Savitch et al., 2001). Hence, it seems very likely that the increased population of QA− due to the altered redox potential of QA and Qb 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°QA− recombination, protecting the Qb 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 1-qO versus 1-qN 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 1-qO relative to 1-qN 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 fulfills 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

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 QA− would decrease the probability for a charge recombination pathway involving P680°Pheo° and will cause stabilization of SQA− pair. In addition, shifting the redox potential of QA toward Qb favors the k2 rate constant and also results in increased steady-state proportion of QA− 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 QA− were found in cold-acclimated Arabidopsis leaves (Fig. 4; Table II). This would ensure that the Qb 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 (Krieger-Liszkay and Rutherford, 1998), and low-temperature-stressed Synechococcus sp. (Sane et al., 2002).

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changes within the acceptor side of PSII involving the redox potentials of QA and QB. We suggest that increasing the free energy gap between P680+ and QA− and narrowing the gap between the redox potential of QA and QB in CH Arabidopsis plants (Fig. 6) result in stabilization of S2QA−. We propose that the increased population of QA− 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 photoindihbition.

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 and 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 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 v.6.0 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-bandwidth 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 S2QA− 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 CO2 conditions as described by Ivanov et al. (1998). Instantaneous (dark) Chl fluorescence at open PSI centers (F0) 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 PSI centers (Fm) 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, qa, and qa Chl fluorescence-quenching parameters were calculated using the procedure described by Quick and Stitt (1989). The fluorescence characteristics were evaluated when the steady-state F0 level was reached.

Photoindihbition 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 QA− 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 QA to QA−. The variable fluorescence decay, reflecting the re-oxidation of QA−, 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 v.6.0 software package (Microcal Software).

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Cold Induced Redox Changes in Photosystem II Acceptor Site


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