Adaptation of photosystem II to high and low light in wild-type and triazine-resistant Canola plants: analysis by a fluorescence induction algorithm

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Abstract Plants of wild-type and triazine-resistant Canola (Brassica napus L.) were exposed to very high light intensities and after 1 day placed on a laboratory table at low light to recover, to study the kinetics of variable fluorescence after light, and after dark-adaptation. This cycle was repeated several times. The fast OJIP fluorescence rise curve was measured immediately after light exposure and after recovery during 1 day in laboratory room light. A fluorescence induction algorithm has been used for resolution and analysis of these curves. This algorithm includes photochemical and photo-electrochemical quenching release components and a photo-electrical dependent IP-component. The analysis revealed a substantial suppression of the photo-electrochemical component (even complete in the resistant biotype), a partial suppression of the photochemical component and a decrease in the fluorescence parameter $F_o$ after high light. These effects were recovered after 1 day in the indoor light.

Keywords Canola (Brassica napus L.) · Chlorophyll fluorescence induction algorithm · Photoinhibition · Reaction kinetics · Triazine-resistance

Abbreviations

- $\beta$ Fraction ($S_0$) of $Q_B$-nonreducing RCs in dark-adapted system
- CET Cyclic electron transport in photosystem I
- Chl Chlorophyll
- $dsq$ Donor side quenching by the secondary electron donor of photosystem II, $Y_{z^+}$
- $F_m$ Maximal fluorescence when degree of primary photochemical—$(q^{PE})$ and of photoelectrochemical quenching $(q^{PE})$ and photoelectroelectric stimulation $(q^{CET}) = 0$, i.e., when $q^{PP} = q^{PE} = q^{CET} = 0$
- $F_m^{STF}$ Maximal fluorescence after single turnover flash
- $F_o$ Minimal fluorescence when $q^{PP} = q^{PE} = q^{CET} = 1$ (see also definition of $F_m$)
- $F_v$ Variable fluorescence $= F - F_o$
- FIA Fluorescence induction algorithm
- $F^{PE}(t)$ Simulated fluorescence emission at time $t$, relative to $F_o$, associated with release of photoelectrochemical quenching at full and invariable primary photochemical quenching $(q^{PP} = 1)$ major contributor of $F_v$ in the O–J - phase
- $F^{PP}(t)$ Simulated fluorescence emission at time $t$, relative to $F_o$, associated with release of primary photochemical quenching at full and invariable photo-electrochemical quenching $(q^{PE} = 1)$ major contributor of $F_v$ in the J–I- phase
- $F^{CET}(t)$ Simulated fluorescence emission at time $t$, relative to $F_o$, associated with photo-electric stimulation at full and invariable primary photochemical- and photo-electrochemical quenching $(q^{PP} = q^{PE} = 1)$ major contributor of $F_v$ in the I–P- phase
- $k_{AB}$ Rate constant of $Q_A^-$ oxidation
- $k_{Hthyl}$ Rate constant of trans-thylakoid proton leak (conductance)
- $k_{IP}$ Rate constant of the simulated photoelectrical stimulation $(F^{CET}(t))$ fitting the major part of the experimental I–P phase
_Introduction_

Plants need light to be able to perform photosynthesis. At the level of individual cells, the light intensity varies in an unpredictable manner. Leaves can adjust to changes in light intensity in various ways. However, when plants are exposed to irradiances that are much higher than those they are adapted to, they use mechanisms to dissipate the excess energy (Prášil et al. 1992; Van Rensen and Curwiel 2000; Tyystjärvi 2008; Takahashi and Badger 2011). If these mechanisms are overloaded, the photosynthetic apparatus becomes damaged, leading to photoinhibition. This phenomenon was first studied by Kok (1956). At present several hypotheses are available with respect to the primary mechanism of the photoinhibitory damage. According to the so called acceptor-side mechanism (Vass et al. 1992) the oxidized primary donor of PSII, P_{680}^{+}, has such a high oxidative potential that it can oxidize pigment molecules if electron transfer from the oxygen evolving complex does not function, this is what sometimes appears to occur. According to the low-light mechanism (Keren et al. 1997) generation of triplet chlorophyll in recombination reactions cause photoinhibition when the electron transport is slow. In the singlet oxygen mechanism (Jung and Kim 1990), photoinhibition is initiated by generation of singlet oxygen by iron-sulfur centers or cytochromes. The last hypothesis, the manganese hypothesis (Hakala et al. 2005), states that release of manganese ion to the thylakoid lumen is the earliest step of photoinhibition. This causes inactivation of the oxygen evolving complex, which leads to damage of PSII via the long-lived P_{680}^{+}. Details and more references on photoinhibition can be found in several reviews: Prášil et al. (1992); Tyystjärvi (2008) and Takahashi and Badger (2011).

Triazine-resistant (R) plants have a mutation in the D1 protein of PSII: at site 264, serine is altered into glycine. Because of this mutation, the R plants are not only unable to bind triazine-type herbicides, but have also a threefold lower rate of electron flow from the primary to the secondary quinone electron acceptor, from the reduced QA to QB (Jansen and Pfister 1990). Thus, the R plants have an intrinsic lower activity of PSII. Furthermore, chloroplasts of resistant plants have shade-type characteristics: more and larger grana, more light harvesting chlorophyll associated with PSII, and a lower chlorophyll a/b ratio (Vaughn and Duke 1984; Vaughn 1986). The combination of shade-type characteristics with a lower electron flow rate from reduced QA to QB leads to lower photochemical quenching and lower energy dependent quenching in the R plants in the light. As a consequence, the R plants are less able to cope with excess light energy, leading to more photoinhibitory damage of the photosynthetic apparatus compared with the sensitive plants, as was reported (Hart and Stemler 1990; Curwiel et al. 1993). The thylakoid membranes of the R chloroplasts have less coupling factor and they utilize the pH gradient less efficiently for photophosphorylation than the triazine-sensitive (S) wild-type plants (Rashid and van Rensen 1987). For a review on triazine-resistance, see van Rensen and de Vos (1992).

Monitoring of chlorophyll a (Chl) fluorescence in intact leaves and chloroplasts is a sensitive non-invasive tool for probing the ongoing electron transport in PS II and for studying the effects of a variety of stressors thereupon (Govindjee 1995; Papageorgiou and Govindjee 2004). We will use the word fluorescence to imply Chl a fluorescence.

It competes with energy trapping (conversion) in photosynthetic reaction centers (RCs) resulting in fluorescence quenching when trapping in the RC is effective (Govindjee 2004). The time pattern of light-induced changes in

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**Symbols and Acronyms**

| Symbol | Definition |
|--------|------------|
| k_L    | Rate constant of the excitation by actinic light |
| k_qbf  | Rate constant associated with accumulation and reduction of Q_B-nonreducing RCs |
| nF_v   | Normalized variable fluorescence (F - F_o)/F_o |
| q^{disq} | Fraction of PSII RCs in which acceptor- and donor-side quenching by Y^+ and Q_A^-, respectively, is released |
| q^{PP} | Degree of primary photochemical quenching with 0 ≤ q^{PP} ≤ 1 |
| q^{PE} | Degree of photoelectrochemical quenching with 0 ≤ q^{PE} ≤ 1 |
| q^{CET} | Degree of photoelectrical F_v stimulation with 0 ≤ q^{CET} ≤ 1 |
| HL     | High light |
| LL     | Low light |
| MTF    | Multi-turnover flash (light pulse) |
| N_{IP} | Integer (0 < N_{IP} < 10) to accommodate delay and steepness of (f^{CET}(t)) response in simulating the variable fluorescence during the I-P- phase |
| OEC    | Oxygen evolving complex |
| PSII (I) | Photosystem II (I) |
| QA     | Primary quinone acceptor of PSII |
| QB     | Secondary quinone acceptor of PSII |
| RC     | Reaction center of PS |
| STF    | Single turnover flash (excitation) |

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fluorescence quenching, often termed fluorescence induction or variable fluorescence, has been measured in a broad time window ranging from μs to several minutes. Here we will focus on those measured in the 10 μs to 2 s time domain. The pattern of variable fluorescence in this time domain is known as the OJIP induction curve of variable fluorescence, where the symbols refer to more or less specific (sub-)maxima or inflections in the induction curve (Strasser et al. 1995; Stirbet et al. 1998; Papageorgiou et al. 2007; Stirbet and Govindjee 2011). The OJ-, JI-, and IP-parts of the curve cover the 0–2.5, 2–20, and 20–300 ms time range, respectively, and can be identified as distinguishable phases of the induction.

The light-dependent Chl a fluorescence yield is variable between a lowest, intrinsic level \( F_0 \) (the “O” level) at full photochemical quenching under dark-adapted conditions and a highest level \( F_m \) (the “P” level) at saturating light intensities at which all quenching is released. Variable fluorescence is defined as \( F_v = F_m - F_0 \). The primary quinone acceptor of PS II, QA, has since long been known as the major and principal quencher; the quenching is released upon its photoreduction (Duysens and Sweers 1963). \( F_m \) is associated with full reduction of QA and with an electron trapping-incompetent closed RC.

The multiphasic recovery kinetics of variable fluorescence after single turnover excitation (STF) has been discussed to point to an energy-linked heterogeneity of RCs and primary processes occurring therein. Kinetic studies have provided evidence for a photochemical role and hitherto unrecognized properties of QB-nonreducing RCs in PS II electron transport (Vredenberg et al. 2006, 2007; Vredenberg 2008; van Rensen and Vredenberg 2009). These data have shown, in contrast to what commonly has been assumed about a photochemical inactivity of QB-nonreducing RCs in PS II electron transport (Melis 1985; Chylla et al. 1987; Lavergne and Leci 1993), that these centers are able to reduce QB after a second hit. The fact that reduced QB-nonreducing RCs (with QA•−) are electron trapping-competent, giving rise to a dark reversible variable fluorescence, has provided evidence that the double-reduced acceptor pair [PheQA]2− in these RCs can reduce QB (Vredenberg et al. 2009).

Quantitative analysis of induction kinetics of variable chlorophyll a fluorescence in intact plant leaves upon 2 s pulses, like we have used here, has enabled the development of a descriptive fluorescence induction algorithm (FIA) (Vredenberg 2008; Vredenberg and Prasil 2009). Briefly, solutions of the differential equations dictated by the electron transfer reaction patterns have provided the mathematical elements of the algorithm with which the kinetics of primary photochemical reactions of PSII can be described quantitatively in terms of their driving forces, rate constants, and transport conductances. The application of the fluorescence induction algorithm (FIA) has provided evidence that the initial events of energy trapping in PSII are accompanied by (i) the release of primary photochemical quenching in a heterogeneous system of QH-reducing and QB-nonreducing RCs during the OJ phase, (ii) the release of photoelectrochemical quenching associated with \( \Delta \mu H \)-controlled accumulation and subsequent double reduction of QB-nonreducing RCs during the JI phase, and (iii) a stimulation of variable fluorescence during the IP-phase by the trans-thylakoid electric potential generated by the CET (PSI) driven proton pump. The Fluorescence Induction Algorithm was successful in fitting many different experimental data obtained in a broad range of conditions and in various plant leaves (Vredenberg and Prášil 2009; Vredenberg 2011).

In this study we have exposed wild-type and triazine-resistant plants of Canola to very high light intensities which caused photoinhibition. After one day the plants were transferred to a laboratory table with much less light. This cycle was repeated several days. The OJIP curve was each time measured after 1 day at high and after low light, respectively. The FIA analysis revealed that the photoelectrochemical component was suppressed after high light (and even completely abolished in the resistant biotype). There was a partial decrease of the photochemical component and a lower fluorescence parameter \( F_0 \) after high light. These effects were recovered after 1 day at the low light of the laboratory.

**Materials and methods**

**Plant material and growth conditions**

Canola (Brassica napus L.) seeds were planted on 18 September in a greenhouse at the University of Queensland, Brisbane, Australia. Sunrise was at about 5 am, sunset at about 6 pm. The roof of the greenhouse was cooled by water. Two plants of wild-type (S) and two of the resistant (R) biotype were used for the measurements. During day-time the temperature varied between 29 and 34°C; the photosynthetic photon flux density (PPFD) varied between 1,100 and 1,200 μmol photons m−2s−1 (HL). The fluorescence measurements were always performed at about 10 am and started on 23 October after the plants were exposed to the high light. After 24 h in the greenhouse the plants were transferred to a table in the laboratory where the temperature varied between 21 and 23°C, and the PPFD was about 8 μmol photons m−2s−1 (LL). The plants were then transferred several times from the laboratory to the greenhouse and back to the laboratory.
Fluorescence measurements

When following the effect of high light in the greenhouse and of low light in the laboratory, the same leaf of each individual plant under investigation was used. Measurements were performed at room temperature between 18 and 20°C. Induction curves of variable chlorophyll fluorescence were measured with a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK) using the standard clip for fixing the leaf in the proper position with respect to the optics of the instrument and kept in the dark for 20 min in the measuring unit. Fluorescence was excited with a 2 s pulse of red light (650 nm) obtained from light-emitting diodes at sub-maximal irradiance of about 280 W m−2 (approximately 1,500 µmol photons m−2s−1). Fluorescence data were recorded at a sampling rate of 10 µs in the lower time range between 0.01 and 0.2 ms, a sampling rate of 0.1 ms between 0.2 and 2 ms, a rate of 1 ms between 2 and 20 ms, and of 10 ms beyond 20 ms. Curves are plotted relative to F0 which is the fluorescence level of the sample in the dark-adapted state. F0 was determined by extrapolation of the model-calculated fluorescence level at t = 0. The experimental traces in general represent the averages of three samples each illuminated once.

The simulation and fitting of the experimental polyphasic fluorescence induction curve with its algorithmic representation FFLA(t) was done with dedicated optimization routines. The fit parameters (rate constants, heterogeneity, fraction, etc.) of the simulation curve FFLA(t) were estimated after application of dedicated routines provided by appropriate software (Mathcad 13, MathSoft, Inc. Cambridge, MA, USA) which calculates the parameter values (vector) for which the least mean square function is estimated after application of dedicated routines provided for energy trapping in semi-closed QB-nonreducing RCs, and kL, kAB, and k2AB are the rate constants of light excitation and of oxidation of the single- and double-reduced primary quinone acceptor QA of PSII, respectively.

Similarly it was shown that the variable fluorescence during the JI phase in the 1–30 ms time range is nearly exclusive due to the release of photoelectrochemical quenching qPE and is in approximation represented by FPE(t) with

\[
F_{\text{PE}}(t) = 1 + nF_v \cdot \left\{ 1 - \frac{1}{\frac{k_{\text{qbf}}}{k_{\text{qbf}} + k_{\text{Hthyl}}} + 1} \cdot \left[ 1 - e^{-k_{\text{IP}} \cdot t} \right] \cdot \frac{k_{\text{qbf}}}{k_{\text{qbf}} + k_{\text{Hthyl}}} \right\}
\]

(2)

in which \( F_{\text{PPsc}}(t) \) is the fraction of semi-closed RCs containing QA (see for definitions and equations Vredenberg and Prasil 2009; Vredenberg 2011), \( k_{\text{qbf}} \) is the rate constant attributed to that of the change in pH at the QA – QB redox side of PSII (related to the actual rate constant of proton pumping by the trans-thylakoid proton pump), and \( k_{\text{Hthyl}} \) the actual passive trans-thylakoid proton leak (conductance). For the experiments presented in this article changes in \( k_{\text{qbf}} \) and \( k_{\text{Hthyl}} \) will be of prime importance to be considered.

Finally the steep variable fluorescence in intact leaves during the IP-phase in the 30–200 ms time range, formerly denoted as \( F_{\text{IP}}(t) \), has been shown to be a response to an electrical field that is generated by the proton pump powered by cyclic electron transport (CET) in PSI and ‘sensed’ by the RCs of PSII (Vredenberg and Prasil 2009; Vredenberg 2011). In anticipation thereupon the variable fluorescence of the IP-phase in the 50–200 ms time, associated with stimulation by CET is termed \( F_{\text{CET}}(t) \) with

\[
F_{\text{CET}}(t) = 1 + \text{IP} \cdot \left[ 1 - e^{-k_{\text{IP}} \cdot t} \cdot \sum_{m=0}^{N_{\text{IP}}} \frac{(k_{\text{IP}} \cdot t)^m}{m!} \right]
\]

(3)

\( \text{IP} \) is the amplitude and \( k_{\text{IP}} \) the rate constant of the fluorescence signal in the IP-phase of the induction response. \( N_{\text{IP}} \) is an integer (5 ≤ \( N_{\text{IP}} \) ≤ 12) to accommodate delay and steepness of the IP-response. \( k_{\text{IP}} \) and \( N_{\text{IP}} \) are related to properties of the CET-driven (PSI) proton pump.

Analysis with fluorescence induction algorithm

It has been shown (Vredenberg and Prasil 2009; Vredenberg 2011) that the variable fluorescence during the OJ phase in the 0.01–1 ms time range is nearly exclusively, if not completely due to the release of primary photochemical quenching \( q^{\text{PP}} \) and is represented by \( F^{\text{PP}}(t) \) with

\[
F^{\text{PP}}(t) = 1 + nF_v \cdot q^{\text{baq}}(t) \cdot \left[ (1 - \beta) \cdot \frac{k_L}{k_L + k_{\text{AB}}} + \beta \cdot (1 + (1 - e^{-k_{\text{IP}} \cdot t}) \cdot e^{-k_{\text{2AB}} \cdot t}) \right]
\]

(1)

in which \( nF_v = (F_{\text{mF}} - F_0)/F_0 \) is the normalized variable fluorescence, \( q^{\text{baq}}(t) = 1 - e^{-k_{\text{qbf}} \cdot t} \). \( \beta \) is the fraction of QA-B-nonreducing RCs, \( \Phi(0 < \Phi < 1) \) is an efficiency factor for energy trapping in semi-closed QB-nonreducing RCs, and \( k_L, k_{\text{AB}}, \) and \( k_{\text{2AB}} \) are the rate constants of light excitation and of oxidation of the single- and double-reduced primary quinone acceptor QA of PSII, respectively.
and P-, are $F(t)$ levels at about 0.01, 1, 30, and 300 ms, respectively, as indicated in the LL-curve. The data show the qualitative effect of the HL treatment of a S-type leaf: (i) a decrease in variable fluorescence at the quasi-steady state $P$-level from $F(t)/F_\infty \sim 5.5$ to $\sim 4$, and (ii) a decline of the O–J and J–I phase in the HL pre-conditioned leaf and less difference in the I–P phase. The thin curves give the comparable curves in an R-type Canola leaf (see Fig. 2).

In Fig. 3 the OJIP curves of the LL-treated R- and S-leaves of Canola are presented. Both curves have been normalized at an equal $P$-level ($F(t)/F_\infty \sim 5.5$) at $t = 200$ ms level with for each $F_\infty = 1$. The curves are similar to those reported for S- and R-type leaves of Chenopodium, except that in the R-type leaf of Canola probably all $Q_B$-nonreducing RCs remain reduced in the dark giving rise to an offset value of $F(t)/F_\infty \sim 1.3$ in the 0.01–0.1 ms time range. The symbols are of the simulation curves calculated with the algorithm (FIA, Eqs. 1–3) for the best fit with the respective experimental curves after low light treatment.

Figure 4 shows, on linear time scales, the simulations of the variable fluorescence responses associated with the release of primary photochemical ($F_{PP}$) and photoelectrochemical quenching ($F_{PE}$), and photoelectric stimulation ($F_{CET}$) of a low (LL) and high light (HL) preconditioned S-type Canola leaf. The curves were obtained after substitution of proper parameter values in Eqs. 1–3 to obtain a best fit of FIA ($=F_{PP} + F_{PE} + F_{CET} - 2$) with the experimental $F_{exp}(t)/F_\infty$ response. The fit and its parameters are shown in Fig. 3 and Table 1, respectively. The fluorescence responses of a type-R leaf measured under identical conditions as in the S-type (Fig. 4) are illustrated in Fig. 5 with corresponding parameter values in the right hand columns of Table I. The low light pre-conditioned R-type Canola leaves show, in comparison with S-type leaves (Table 1, Figs. 3 and 5) and in agreement with results reported for other plant species (van Rensen and Vredenberg 2009) a lower rate of $Q_A^-$ oxidation ($k_{AB}$) and a higher concentration of $Q_B$-nonreducing RCs ($\beta$).
As shown in Table 1, R-type leaves have, in addition, a higher thylakoid proton conductance ($k_{Hthyl}$).

The data collected in Table 1 and Figs. 4 and 5 show clear effects of high light treatment on Canola leaves. Using FIA, these effects can be quantified in terms of changes in: (i) 9–16% decrease in $F_o$, (ii) 22–32% decrease in the normalized variable fluorescence ($nF_v$) associated with full reduction of the primary quinone electron acceptor QA and equivalent with a decrease in PSII primary photochemical efficiency (from $\Phi_{pp} = [nF_v/(nF_v + 1)] \sim 0.7$ towards $\sim 0.6$), (iii) a substantial increase in basal proton conductance of the thylakoid membrane ($k_{Hthyl}$), notably 8- and 30-fold in S- and R-type leaves, respectively, and associated with 65 and 100% suppression, respectively, of the release of photo-electrochemical quenching $q_{PE}(t)$, and (iv) a decrease in the steepness of the potential-driven stimulation of variable fluorescence ($F_{CET}(t)$), quantified by $N_{IP}$ (last row in Table 1).

The variable fluorescence curves of the respective S- and R-type Canola leaves at the end of a 4 (6) day period with 2 (3) subsequent LL- and HL treatments were found to be qualitatively similar to those at the start of the period (data not shown). This indicates a reasonable and reversible stability of the system during and after the alternating light

Table 1  Kinetic parameters (rate constants (ms$^{-1}$), amplitudes, fractions, curve steepness) of the closest fit $F(t)$ using the fluorescence induction algorithm (FIA, Eqs. 1–3) with experimental OJIP variable fluorescence curves $F^{exp}(t)$ measured during a light pulse of $\sim 1,500$ μmol photons m$^{-2}$s$^{-1}$ in low- (LL) and high light (HL) pre-conditioned atrazine-susceptible (S) and -resistant (R) Canola leaves

| Parameters | S-type | R-type |
|------------|--------|--------|
|            | LL     | HL     | LL     | HL     |
| $F_{o}$ at 0.1 ms (a.u.) ($F_o$) | 660    | 550    | 1,125  | 1,025  |
| Rate constant light excitation ($k_L$) | 1.4    | 1.4    | 2.3    | 2.3    |
| Rate constant qPE-release ($k_{qbf}$) | $9 \times 10^{-2}$ | $1 \times 10^{-1}$ | $9 \times 10^{-2}$ | $9 \times 10^{-2}$ |
| Rate constant QA$^-$ oxidation ($k_{AB}$) | 1.9    | 2.2    | 0.8    | 1.6    |
| Rate constant QA$^2$ oxidation ($k_{2AB}$) | $5 \times 10^{-2}$ | $5 \times 10^{-2}$ | $7.5 \times 10^{-2}$ | $8 \times 10^{-2}$ |
| Rate constant conductance leakage ($k_{Hthyl}$) | $1.5 \times 10^{-2}$ | $1.2 \times 10^{-1}$ | $3 \times 10^{-2}$ | $9 \times 10^{-1}$ |
| Fraction QB-nonreducing RCs ($b$) | 0.13   | 0.13   | 0.27   | 0.35   |
| Efficiency e-trapping donor side ($\bar{\theta}$) | 0.3    | 0.3    | 0.3    | 0.3    |
| Normalized variable fluorescence ($nF_v$) | 2.3    | 1.8    | 2.2    | 1.5    |
| Amplitude IP rise ($F_{CET}(IP)$) | 2.17   | 1.4    | 1.1    | 0.5    |
| Rate constant IP rise ($k_{IP}$) | $1 \times 10^{-1}$ | $1.1 \times 10^{-1}$ | $1.4 \times 10^{-1}$ | $8 \times 10^{-2}$ |
| Steepness IP rise ($N_{IP}$) | 8      | 5      | 8      | 3      |
protocol that was followed. A comparison of the FIA-parameters shows a small attenuation effect in parallel with the duration of the period (data not shown). This effect is most pronounced for the decrease in the magnitude of the variable fluorescence FPE associated with the release of photo-electrochemical quenching as reflected by the increase in the thylakoid proton conductance ($k_{\text{Hthyl}}$).

**Discussion**

Carr and Björk (2007) acclimated thalli of Ulva fasciata for a long time to a low light intensity (80 μmol photons m$^{-2}$s$^{-1}$) and then exposed them to prolonged high irradiance (1,500 μmol photons m$^{-2}$s$^{-1}$) followed by recovery at the low irradiance. They observed that under the...
photoinhibitory high light the D1 protein degraded rapidly and that the non-photochemical quenching of chlorophyll fluorescence decreased following the same trend. Both reached a low steady state level after about 100 min and recovered fast after thalli were transferred to the low irradiance of 80 µmol photons m⁻² s⁻¹. The initial values after 200 min were substantially higher than for the S leaf; the J-level was lower in the R biotype which is in agreement with many other reports (e.g., Jansen and Pfister 1990). It causes a slower re-oxidation of the acceptor side of PSII resulting in a higher fluorescence emission in the 1–2 ms time region (J-level). A higher fraction of Qb-nonreducing centers in R plants has been reported earlier (van Rensen and Vredenberg 2009). The higher excitation rate kL agrees with the reported shape-type chloroplasts of the resistant plants (having more light harvesting chlorophyll connected with PSII) (Vaughn and Duke 1984; van Rensen and Curwiel 2000). The higher basic proton conductance kHthyl is in accordance with the finding by Rashid and van Rensen (1987) that the thylakoids of the R chloroplasts utilize the pH gradient less efficiently for photophosphorylation than the thylakoids of the wild-type (S) plants.

Comparing the parameters of leaves pre-conditioned at high (HL) or low (LL) light intensity, it appears that after HL pre-conditioning, the QA⁻ oxidation, kAB, and the basic proton conductance, kHthyl, were higher. Fv, normalized variable fluorescence, nFv, and the steepness of the IP rise, NIP, were lower after HL pre-conditioning.

Pre-conditioning at HL leads to photoinhibition of the plants and degradation of the D1 protein (e.g., Carr and Björk 2007). Apparently, damage to the D1 protein causes an increase of the rate of electron transport between QA and Qb. The higher proton conductance kHthyl (Table 1) is probably due to damage to the thylakoid membranes caused by photoinhibition leading to proton leakage. The lower value of nFv indicates a lower photochemical quenching and consequently a lower primary photochemical efficiency of PSII in the HL pre-conditioned plants. The lower steepness of the IP rise, NIP, maybe related to a slower increase of a pH gradient, caused by a higher proton conductance in the HL plants.

Comparisons of the curves analyzed at different linear time scales (Fig. 4 for Canola S-type leaves, and Fig. 5 for R-type ones) allow the following conclusions on the effect of LL and HL on each of the individual components of variable fluorescence. The release of primary photochemical quenching FP (Eq. 1, left hand figures) governs variable fluorescence in time range up to 2 ms; that of photoelectrochemical quenching FP(Eq. 2, middle figures) predominates in the range between 2 and 50 ms; and that ascribed to photoelectrochemical stimulation FCET (Eq. 3, right hand figures) is responsible for the changes in the
20–300 ms range. After photoinhibition (HL pre-conditioning) the plants showed less release of photochemical quenching, probably due to damaged D1 protein. The middle figures of Figs. 4 and 5 show that HL treatment decreases the release of \( q_{PE} \). The fact that HL treatment also decreases the non-photochemical quenching (NPQ) (Carr and Björk 2007) confirms strongly a relation between NPQ and photoelectrical quenching (Vredenberg 2011). Also the variable fluorescence emission associated with release of photoelectrochemical quenching was less after HL treatment; in the R plant it even became zero. This indicates that the electrochemical potential of protons becomes lower after HL treatment, possibly due to damage to the thylakoid membrane associated with photoinhibition. The \( F_{CEF} \) components illustrate the release of quenching due to the proton potential build up by cyclic electron transport (Vredenberg 2011). After HL treatment, this release of quenching was decreased in the R plants, while it was increased in the S plants. The reason for this discrepancy is as yet unknown.

The pre-conditioning at high light for a full day was followed by adaptation at very low light, also for a full day. This cycle was repeated three times. The measurements presented are from the first day (after adaptation at high light) and from the second day (after 1 day at low light). The measurements of the second and third cycle were found to be qualitatively similar to those of the first 2 days. This indicates a reversible stability of the system during and after the alternating light protocol that was followed.

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