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

β 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^{PP}) 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

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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^{dsq}	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 $0 \leq q^{PP} \leq 1$
q^{PE}	Degree of photoelectrochemical quenching with $0 \leq q^{PE} \leq 1$
q^{CET}	Degree of photoelectrical F_v stimulation with $0 \leq q^{CET} \leq 1$
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)
Q_A	Primary quinone acceptor of PSII
Q_B	Secondary quinone acceptor of PSII
RC	Reaction center of PS
STF	Single turnover flash (excitation)

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) reduction of the plastoquinone pool promotes double reduction, protonation, and loss of the primary quinone electron acceptor of photosystem II (PSII), Q_A . In this situation, recombination reactions between Q_A^- and P_{680}^+ can lead to the formation of triplet chlorophyll, that may react with oxygen to produce harmful singlet oxygen. In the donor-side mechanism (Callahan et al. 1986; Anderson

et al. 1998) 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 Q_A to Q_B (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 Q_A to Q_B 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

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_o (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 $F_v = F_m - F_o$. The primary quinone acceptor of PS II, Q_A , 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 Q_A 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 Q_B -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 Q_B -nonreducing RCs in PS II electron transport (Melis 1985; Chylla et al. 1987; Lavergne and Leci 1993), that these centers are able to reduce Q_B after a second hit. The fact that reduced Q_B -nonreducing RCs (with Q_A^-) are electron trapping-competent, giving rise to a dark reversible variable fluorescence, has provided evidence that the double-reduced acceptor pair $[\text{Phe}Q_A]^{2-}$ in these RCs can reduce Q_B (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 Q_B -reducing and Q_B -nonreducing RCs during the OJ phase, (ii) the release of photoelectrochemical quenching associated with $\Delta\mu\text{H}$ -controlled accumulation and subsequent double reduction of Q_B -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 Prasil 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_o 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 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (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 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (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 F_o , which is the fluorescence level of the sample in the dark-adapted state. F_o 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 $F^{\text{FIA}}(t)$ was done with dedicated optimization routines. The fit parameters (rate constants, heterogeneity, fraction, etc.) of the simulation curve $F^{\text{FIA}}(t)$ 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 minimal, where NN is the number of data points (in most experiments $NN \geq 50$). Reduction of data points was in some cases purposely applied for $F^{\text{FIA}}(t)$ curves to facilitate better comparison with the experimental curve $F^{\text{exp}}(t)$.

Analysis with fluorescence induction algorithm

It has been shown (Vredenberg and Prášil 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^{PP} and is represented by $F^{\text{PP}}(t)$ with

$$F^{\text{PP}}(t) = 1 + nF_v \cdot q^{\text{dsq}}(t) \cdot [(1 - \beta) \cdot \frac{k_L}{k_L + k_{AB}} + \beta \cdot (1 + (1 - e^{-\phi k_L t}) \cdot e^{-k_{2AB} t})] \quad (1)$$

in which $nF_v (=F_m^{\text{STF}} - F_o)/F_o$ is the normalized variable fluorescence, $q^{\text{dsq}}(t) = 1 - e^{-k_L t}$, β is the fraction of Q_B-nonreducing RCs, Φ ($0 \leq \Phi < 1$) is an efficiency factor

for energy trapping in semi-closed Q_B-nonreducing RCs, and k_L , k_{AB} , and k_{2AB} are the rate constants of light excitation and of oxidation of the single- and double-reduced primary quinone acceptor Q_A 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 q^{PE} and is in approximation represented by $F^{\text{PE}}(t)$ with

$$F^{\text{PE}}(t) = 1 + nF_v \cdot \{ [1 - f^{\text{PPsc}}(t)] \cdot [1 - e^{-k_{\text{qbf}} t}] \cdot \frac{k_{\text{qbf}}}{k_{\text{qbf}} + k_{\text{Hthyl}}} + 1 \} \cdot [1 - e^{-k_{\text{qbf}} t}] \cdot \frac{k_{\text{qbf}}}{k_{\text{qbf}} + k_{\text{Hthyl}}} \quad (2)$$

in which $f^{\text{PPsc}}(t)$ is the fraction of semi-closed RCs containing Q_A⁻ (see for definitions and equations Vredenberg 2011), k_{qbf} is the rate constant attributed to that of the change in pH at the Q_A – Q_B redox side of PSII (related to the actual rate constant of proton pumping by the trans-thylakoid proton pump), and k_{Hthyl} the actual passive trans-thylakoid proton leak (conductance). For the experiments presented in this article changes in k_{qbf} and k_{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 Prášil 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

$$F^{\text{CET}}(t) = 1 + \text{IP} \cdot \left[1 - e^{-k_{\text{IP}} t} \cdot \sum_{m=0}^{N_{\text{IP}}} \frac{(k_{\text{IP}} \cdot t)^m}{m!} \right] \cdot \frac{k_{\text{IP}}}{k_{\text{IP}} + k_{\text{Hthyl}}} \quad (3)$$

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

Results

Figure 1 shows the response of variable fluorescence in dark-adapted high greenhouse light (HL) and in low laboratory light (LL) pre-conditioned S-type Canola leaves upon excitation with a light pulse of ~1,500 μmol photons m⁻²s⁻¹ intensity plotted with normalization to $F(t)$ at 10 μs (F_o) on a log time scale from 10 μs to 1 s. O-, J-, I-,

Fig. 1 Variable fluorescence curves in low (LL) and high light (HL) pre-conditioned atrazine-susceptible (S-type) Canola leaf upon exposure to a light pulse of $\sim 1,500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ intensity. Curves are plotted with normalization to $F(t)$ at $10 \mu\text{s}$ (F_0) on a log time scale from $10 \mu\text{s}$ to 1 s . O-, J-, I-, and P-, are $F(t)$ levels at about 0.01 , 1 , 20 , and 200 ms , respectively, as indicated in top curve. The thin curves are the comparable curves in an R-type Canola leaf (see Fig. 2)

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 $F(t)/F_0 \sim 5.5$ to ~ 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 responses of an R-type leaf. The effect of HL in a R-type leaf is illustrated in Fig. 2 with a comparatively stronger depression of the JI phase. The thin curves are those of the S-type leaf of Fig. 1.

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 ($F(t)/F_0 \sim 5.5$) at $t = 200 \text{ ms}$ level with for each $F_0 = 1$. 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 $F(t)/F_0$ (~ 1.3) in the

Fig. 2 Same fluorescence curves as in Fig. 1 for low (LL) and high light (HL) pre-conditioned atrazine-resistant (R-type) Canola leaf. The thin curves are the comparable curves in an S-type Canola leaf

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 ($=F^{\text{PP}} + F^{\text{PE}} + F^{\text{CET}} - 2$) with the experimental $F^{\text{exp}}(t)/F_0$ 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 (β).

Fig. 3 Variable fluorescence induction curves F^{exp} (same as in Figs 1 and 2) of dark-adapted S- and R-type LL pre-conditioned Canola leaves upon a light pulse of $\sim 1,500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ intensity plotted on a log time scale (dashed lines). Symbols are of the simulated curves $F^{\text{FIA}}(t)$ calculated with the equations for the OJIP response in the 0–1 s time range, given in the text (Eqs. 1–3). Values of the matching parameters are given in the third and fourth (S-type) and the fifth and sixth (R-type) column of Table 1

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

Parameters	S-type LL	HL	R-type LL	HL
F^{exp} at 0.1 ms (a.u.) (F_0)	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})	$9 \cdot 10^{-2}$	$1 \cdot 10^{-1}$	$9 \cdot 10^{-2}$	$9 \cdot 10^{-2}$
Rate constant Q_A^- oxidation (k_{AB})	1.9	2.2	0.8	1.6
Rate constant Q_A^{2-} oxidation ($k_{2\text{AB}}$)	$5 \cdot 10^{-2}$	$5 \cdot 10^{-2}$	$7.5 \cdot 10^{-2}$	$8 \cdot 10^{-2}$
Rate constant conductance leakage (k_{Hthyl})	$1.5 \cdot 10^{-2}$	$1.2 \cdot 10^{-1}$	$3 \cdot 10^{-2}$	$9 \cdot 10^{-1}$
Fraction Q_B -nonreducing RCs (β)	0.13	0.13	0.27	0.35
Efficiency e -trapping donor side (ϕ)	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)	0.8	1.2	1.1	0.5
Rate constant IP rise (k_{IP})	$1 \cdot 10^{-1}$	$1.1 \cdot 10^{-1}$	$1.4 \cdot 10^{-1}$	$8 \cdot 10^{-2}$
Steepness IP rise (N_{IP})	8	5	8	3

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_0 (ii), 22–32% decrease in the normalized variable fluorescence (nF_v) associated with full reduction of the primary quinone electron acceptor Q_A and equivalent with a decrease in PSII primary photochemical efficiency (from $\phi_{\text{pp}} [=nF_v/(nF_v + 1)] \sim 0.7$ towards ~ 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 $q^{\text{PE}}(t)$, and (iv) a decrease in the steepness of the potential-driven stimulation of variable fluorescence ($F^{\text{CET}}(t)$), 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

Fig. 4 Time courses (from left to right panel) of the FIA-constituent components $F^{PP}(t)$, $F^{PE}(t)$ and $F^{CET}(t)$ that quantify the release of photochemical- (q^{PP}), photoelectrochemical (q^{PE}) quenching and photoelectric fluorescence stimulation (q^{CET}), respectively, in a low (LL, full symbols) and high light (HL, open symbols) pre-conditioned S-type Canola leaf. Curves are on a linear time scale (note the

difference in scales in the panels) and were calculated with the fluorescence induction algorithm (FIA, Eqs. 1–3) for parameters listed in the respective columns in Table 1. The sum (minus 2) of the curves is the best fit for the experimental curve (see Fig. 3). Full symbols are from LL pre-conditioned leaves; HL pre-conditioned leaves are shown as open symbols

Fig. 5 Same as Fig. 4 for low (LL) and high light (HL) pre-conditioned R-type Canola leaf

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_{Hthyl}).

Discussion

Carr and Björk (2007) acclimated thalli of *Ulva fasciata* for a long time to a low light intensity ($80 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and then exposed them to prolonged high irradiance ($1,500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) 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 $80 \mu\text{mol photons m}^{-2}\text{s}^{-1}$: to about 60% of the original values after 200 min at $80 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. Our experiments aimed at a different goal. We exposed plants during a full day at a high light intensity and then transferred them to very low intensity, again for a full day; and repeated this cycle several times. We measured fast fluorescence induction changes in an adapted steady state situation.

Many earlier studies (reviewed by Tyystjärvi (2008) and Takahashi and Badger (2011)) were aimed at different effects of photoinhibition: on its mechanism, on the structure of PSII, damage and repair of PSII, and mechanisms of dissipation of excess light energy. This article deals with adaptation of plants to high and low light conditions. The fast fluorescence induction curves were measured up to 2 s, and the transients were analyzed by a fluorescence induction algorithm.

In Fig. 1 the OJIP fluorescence transients are presented for Canola leaves under different conditions. The full bold curve represents the variable fluorescence for a wild-type (S) leaf pre-conditioned at low light (LL, $8 \mu\text{mol photons m}^{-2}\text{s}^{-1}$). It shows the usual transients O, J, I, and P, as reported earlier for intact leaves under comparable conditions (Strasser et al. 1995; van Rensen and Vredenberg 2009). The dashed bold curve is measured after pre-conditioning at high light (HL, between 1,100 and 1,200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). While the curves were measured for the same leaf, the J, I, and P transients after pre-conditioning at HL were all lower than after pre-conditioning at LL. The thin lines represent the comparable curves for a triazine-resistant (R) leaf. In Fig. 2, the bold lines show the measurements for an R leaf, pre-conditioned at LL (full line) or at HL (dashed line) and the thin curves are the measurements for the S leaf. As was found in the S leaf, in the R leaf the J, I and P transients after pre-conditioning after HL were also lower than after pre-conditioning at LL. As can be observed in Figs 1 and 2, F_o for the R leaf was substantially higher than for the S leaf; the J-level was comparatively more and the I-transient was less pronounced in the low light-adapted R leaf. This has been observed earlier by Kohno et al. (2000) and van Rensen and Vredenberg (2009). The higher F_o in the R leaf is ascribed to a larger fraction of dark-reduced Q_B -nonreducing reaction centers; the higher J-level in R can be explained by the lower rate of electron flow between Q_A and Q_B (Jansen and Pfister 1990).

In Fig. 3 the results are presented of a simulation of the curves of LL pre-conditioned S and R leaves using the

algorithm as described in Eqs. 1–3. The diamonds of the calculations fit the dashed lines of the measurements very well. The parameters used to obtain the best fit are presented in Table 1. There are differences between these kinetic parameters. In low light-adapted S and R leaves, F_o , excitation rate k_L , basic proton conductance k_{Hthyl} , and the fraction of Q_B -nonreducing centers β were substantially higher in the R-type. The parameter of Q_A^- oxidation, k_{AB} , 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 Q_B -nonreducing centers in R plants has been reported earlier (van Rensen and Vredenberg 2009). The higher excitation rate k_L 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 conduction k_{Hthyl} 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 Q_A^- oxidation, k_{AB} , and the basic proton conductance, k_{Hthyl} , were higher. F_o , normalized variable fluorescence, nF_v , and the steepness of the IP rise, N_{IP} , 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 Q_A and Q_B . The higher proton conductance k_{Hthyl} (Table 1) is probably due to damage to the thylakoid membranes caused by photoinhibition leading to proton leakage. The lower value of nF_v 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, N_{IP} , 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 F^{PP} (Eq. 1, left hand figures) governs variable fluorescence in time range up to 2 ms; that of photoelectrochemical quenching F^{PE} (Eq. 2, middle figures) predominates in the range between 2 and 50 ms; and that ascribed to photoelectric stimulation F^{CET} (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^{CET} 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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