Photosynth Res (2009) 101:217–232
DOI 10.1007/s11120-009-9451-1

REVIEW

Delayed fluorescence in photosynthesis

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Received: 22 November 2008 / Accepted: 27 May 2009 / Published online: 23 June 2009

Abstract Photosynthesis is a very efficient photochemical process. Nevertheless, plants emit some of the absorbed energy as light quanta. This luminescence is emitted, predominantly, by excited chlorophyll *a* molecules in the light-harvesting antenna, associated with Photosystem II (PS II) reaction centers. The emission that occurs before the utilization of the excitation energy in the primary photochemical reaction is called prompt fluorescence. Light emission can also be observed from repopulated excited chlorophylls as a result of recombination of the charge pairs. In this case, some time-dependent redox reactions occur before the excitation of the chlorophyll. This delays the light emission and provides the name for this phenomenon—delayed fluorescence (DF), or delayed light emission (DLE). The DF intensity is a decreasing polyphasic function of the time after illumination, which reflects the kinetics of electron transport reactions both on the (electron) donor and the (electron) acceptor sides of PS II. Two main experimental approaches are used for DF measurements: (a) recording of the DF decay in the dark after a single turnover flash or after continuous light excitation and (b) recording of the DF intensity during light adaptation of the photosynthesizing samples (induction curves), following a period of darkness. In this paper we review historical data on DF research and recent advances in the understanding of the relation between the delayed fluorescence and specific reactions in PS II. An experimental method for simultaneous recording of the induction transients of prompt and delayed chlorophyll fluorescence and decay curves of DF in the millisecond time domain is discussed.

Keywords Delayed (chlorophyll) fluorescence · Chlorophyll fluorescence · Photosystem II (PS II) · Reaction center · Charge recombination · Electron transport

Abbreviations

Chl^*	Excited state of a chlorophyll molecule
DF	Delayed (chlorophyll) fluorescence
ET	Electron transport
F_o	Fluorescence intensity when all the reaction centers are assumed to be open, at the "O" level
F_j	Fluorescence intensity at ~3 ms
F_i	Fluorescence intensity at ~30 ms
F_p	Maximal measured fluorescence intensity, at the "P" level
IC	Induction curve
L	Delayed fluorescence intensity
P_{700}	Primary (chlorophyll) electron donor of PS I
P_{680}	Primary (chlorophyll) electron donor of PS II
Pheo	Pheophytin on D1 protein of PS II
PF	Prompt (chlorophyll) fluorescence
PQ	Plastoquinone

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PS	Photosystem
Q_A	Primary quinone (electron) acceptor of PS II
Q_B	Secondary quinone (electron) acceptor of PS II
RC	Reaction center
Z (also called Y_z)	Secondary (electron) donor in PS II, Tyr-161 of the D1 protein of PS II

Introduction

Delayed (chlorophyll) fluorescence (DF) is light that is emitted from green plants, algae and photosynthesizing bacteria in the red-infra-red region of the spectrum for a short time after they have been exposed to light, but after the prompt fluorescence has decayed. The source of this emission is either chlorophyll *a*, or bacteriochlorphyll molecule, depending upon the organism used. In the specialized literature, this phenomenon is described by several terms, each reflecting its specific characteristics—e.g., delayed fluorescence, delayed luminescence, delayed light emission, and afterglow. This phenomenon was discovered by Strehler and Arnold (1951) when they were attempting to use the firefly luminescence for the measurement of the light-induced accumulation of ATP in the green alga *Chlorella*; the authors discovered that even without the addition of luciferase and luciferin, there was a long-lived glow from algal cells and chloroplasts in darkness following illumination. The observed DF was characteristic of different photosynthesizing samples used—leaves (Strehler and Arnold 1951), chloroplasts and photosynthesizing bacteria (Arnold and Thompson 1956).

For more than half a century this amazing property of photosynthetic systems has attracted the interest of many researchers (see e.g., Shuvalov and Litvin 1969; Barber and Kraan 1970; Wraight and Crofts 1971; Itoh and Murata 1973; Hipkins and Barber 1974; Jursinic and Govindjee 1977, 1982; Malkin and Barber 1978; Grabolle and Dau 2005). Several outstanding reviews have appeared that discuss the internal relationship between DF and the storage of light energy in photosynthesis (see, e.g., Lavorel 1975; Amesz and Van Gorkom 1978; Malkin 1979; Lavorel et al. 1982; Jursinic 1986; Veselovskii and Veselova 1990; Gaevsky and Morgun 1993; Radenovic et al. 1994; Tyystjarvi and Vass 2004). But even now, the full picture of the phenomenon remains unclear. There are two main reasons for this: (a) photosynthesis is a very complicated process that includes a multitude of simultaneous reactions, and DF is correlated with many of these reactions in a specific way; (b) it is technically difficult to measure DF because its spectrum is the same as that of prompt fluorescence (PF), but its intensity is lower by at least two orders of magnitude. As DF is emitted for a long time after the disappearance of PF, measurement of the two types of light emission is based only on their temporal separation. For measurement of DF, each laboratory usually constructs its own unique equipment, which as a rule has considerably different characteristics from the equipment of other laboratories. For this reason, even when the same sample is used, researchers often find differences in their results. Comparison of such results is difficult and sometimes leads to incorrect interpretations. In this review, we summarize contemporary ideas about the mechanism(s) of DF and its relationship with different photosynthetic reactions and present basic ideas for the understanding of this phenomenon for beginning researchers. The methodical approaches for the measurement of DF are also discussed. Our aim is to give the basic know-how about delayed fluorescence and to initiate attempts for integrating this potentially highly informative method with other methods in use (see Amesz and Hoff 1996; Aartsma and Matysik 2008). We hope that simultaneous measurements with several biophysical techniques would provide a powerful means for a complete understanding of the photosynthetic process in vivo.

Origin of delayed fluorescence

Delayed fluorescence is emitted predominantly from Photosystem (PS) II (Jursinic 1986). In mutant algae in which PS II is missing, DF is very weak or not observed at all. Light emission from PS II particle suspensions is 60–90 times more intense compared to that from PS I suspensions (Amesz and Van Gorkom 1978). This is why in this review delayed fluorescence will be discussed in terms of PS II.

The similarity between the emission spectra of delayed and prompt chlorophyll fluorescence (PF) (Arnold and Thompson 1956; Clayton 1969; Sonneveld et al. 1980a; Grabolle and Dau 2005) shows that in both cases the photon is a result of the radiative deactivation of the singlet excited state of the PS II antenna chlorophyll (Chl) *a* (Krause and Weis 1991; Lang and Lichtenthaler 1991).

The PF emission is practically extinguished about 5 ns after the light is switched off (Jursinic 1986; Krause and Weis 1991); its intensity decays polyphasically with characteristic lifetimes that range from several ps to 2 ns (Miloslavina et al. 2006). On the contrary, DF has components that decay in very different time domains: in the nanoseconds (Christen et al. 2000), microseconds (Jursinic and Govindjee 1977; Jursinic et al. 1978; Wong et al. 1978; Christen et al. 1998; Mimuro et al. 2007; Buchta et al. 2008), milliseconds (Hipkins and Barber 1974; Barber and Neumann 1974; Zaharieva and Goltsev 2003; Goltsev et al.

2005; Buchta et al. 2007), seconds (Rutherford et al. 1984; Hideg et al. 1991; Katsumata et al. 2008), and even in the hour time range (Hideg et al. 1990).

The different lifetimes of PF and DF show that the mechanism of the formation of the excited singlet state of the PS II chlorophyll antenna pigment that produces the emission is different for PF and DF. In the case of PF, chlorophyll is excited either via direct absorption of light, or by fast energy transfer from other chlorophyll molecules. In DF the excited antenna chlorophyll is formed as a result of back electron transfer and charge recombination in the reaction center of PS II, followed by repopulation of the excited chlorophyll (Chl^*) state of the PS II antenna system by fast energy transfer (Arthur and Strehler 1957). Delayed fluorescence exists because each of the redox reactions of the photosynthetic electron transport is reversible. The absorption of a light quantum leads to the formation of Chl^* state, energy transfer to P_{680} and charge separation between P_{680} and Pheo. The separated charges in the couple $P_{680}^+Pheo^-$ however can recombine, which will lead to P_{680}^* formation. The rapid exciton equilibration (Dau and Sauer 1996) delocalizes the excitation among about 200 PS II antennae chlorophylls, including the light-harvesting complexes (denoted as $Chl^*_{200}$by Grabolle and Dau (2005)), which can emit fluorescence. This emission decays with a lifetime of 2–4 ns and in theory already can be considered as the fastest-decaying component of delayed fluorescence. In practice this component is considered a variable fluorescence, which is a part of PF (Schatz et al. 1988), because its short life-time makes its separation from PF impossible.

In fact, all electron transfer reactions in the reaction center of PS II—in its donor as well as in its acceptor side, are reversible. Depending on the "location" in electron transport chain from which the electron returns, the DF emission is delayed with different times after the absorption of the photon that had originally induced the primary charge separation. The charge recombination which is a result of back electron transport from different PS II electron carriers can lead to the formation of "secondary excited" Chl* and DF emission with much longer lifetimes (micro-, milliseconds and even seconds). Because of the decreasing probability of this "long distance" reversal of the electron transport, the intensity of the emitted DF will decrease with the distance between the separated charges. With time, as the charge couples that can recombine start to disappear, the DF intensity decreases. This is the reason why in dark, after illumination by a single light pulse or by continuous light, the DF decay can be observed. The time course of the DF dark decay is described by a generally decreasing multi-component function in which all the different DF decay components overlap. This decay is first monotonous, but, under certain conditions, may form a transient maximum in the seconds time range (Desai et al. 1983; Hideg et al. 1991).

Each DF decay component is a result of reversal of electron transfer reactions in a specific state of PS II, for example $S_3Z^+Q_A^-Q_B$, or $S_3Z^+Q_A^-Q_B^=$. The lifetime of different DF components is determined by the lifetime of the corresponding PS II state. The possibility to use DF for the investigation of photosynthesis depends on the correct identification of the different DF components and their connection to the specific reactions that occur in the photosynthetic machinery (Jursinic 1986; Tyystjarvi and Vass 2004; Goltsev et al. 2005).

The initial parts of the DF dark decay curve (the region of the fastest decay) can be represented by a sum of exponential functions (Lavorel 1975). The second and tens-of-second regions of the DF dark decay curve, however, cannot be described with sufficient accuracy as a sum of exponential functions. In these regions the curve is better described by a hyperbolic function, which can be explained by the simultaneous participation of many electron-transfer reactions, each having a different rate (Lavorel 1975), or with the heterogeneity of the system with respect to the depth of the energy traps, which is characterized by a normal distribution of the ΔG^* values (Lavorel and Dennery 1982; Tyystjarvi and Vass 2004).

Three types of processes can determine the kinetics of the DF dark decay:

(1) Redox reactions in which one of the separated charges takes part; these reactions result in the decrease of the concentration of charge couples; in this case the luminescence is referred to as "leakage" type. This mechanism of DF decay explains mainly the fastest DF components (micro- and sub-millisecond time range).
(2) Charge recombination reactions, resulting in the formation of secondarily excited states and DF quanta emission; this is "deactivation" type luminescence (Lavorel 1975). This process contributes to the slower DF components (milliseconds and longer). In both (1) and (2) cases, the DF intensity decreases because of a decrease in the concentration of the separated charge couples.
(3) A third type of processes affect the kinetics of DF dark decay by changing the rate constant of recombination of charge couples, and correspondingly the DF quantum yield. These processes are related to the dark deactivation of the energized state of the thylakoid membrane ($\Delta\mu H^+$ or $\Delta\Psi$).

When the reactions that determine the dark decay are of the first order (as it is for the leakage type DF, where the disappearance of only one of the separated charges is responsible for the decrease of the concentration of the DF

emitting state), then the DF relaxation curve can be described as a sum of exponential functions, where the kinetic components differ in lifetime (τ) and amplitude (L):

$$L(t) = \sum_i L_i e^{-t/\tau_i}, \quad (1)$$

where, $L(t)$ is DF emitted at time t after the light is switched off; L_i is the amplitude of the i-th component, and τ_i is its characteristic lifetime. With this model, the deactivation type of DF can also be described, when the separated charges remain in the same protein complex, as is for example for the PS II state $Z^+P_{680}Q_A^-$.

The PS II redox states that mainly generate DF are $P_{680}^+Pheo^-$, $P_{680}^+Q_A^-$, $Z^+Q_A^-$, $Z^+Q_B^-$ and $S_iZQ_B^-$. Delayed fluorescence, emitted in the micro- and millisecond time domain, is mostly related to $Z^+Q_A^-$ state of PS II. For an ensemble of PS II, the actual population of the Chl*-state (fraction of PS II in the Chl*-state denoted as $[Chl^*]$) is assumed to be determined by the free-energy difference between the excited-antenna state and the radical-pair state of PS II reaction center, $Z^+Q_A^-$, reached at this time according to:

$$[Chl^*]/[Z^+Q_A^-] = \exp\left(\Delta G^*_{Z^+Q_A^-}/k_BT\right), \quad (2)$$

where, $\Delta G^*_{Z^+Q_A^-} = \Delta G^*_3$ (see Fig. 1) represents the difference in the Gibbs free energy ($\Delta G^*_3 < 0$) between the excited-antenna state (Chl*) and the PS II radical-pair state ($Z^+Q_A^-$), k_B is the Boltzmann constant, and T is the absolute temperature in Kelvin.

Consequently the amount of DF quanta emitted from this state (L) is given by:

$$L = c\varphi_f[Chl^*] = c\varphi_f[Z^+Q_A^-] \cdot \exp\left(\Delta G^*_3/k_BT\right), \quad (3)$$

where, c is a proportionality coefficient that depends on the amount of active reaction centers of PS II in the sample and on the equipment constant that is used to measure the DF (this coefficient connects the value that is monitored by the equipment, in volts, with the actual number of emitted DF quanta); φ_f is the quantum yield for fluorescence emission of the antenna chlorophylls.

Delayed fluorescence of PS II, measured few milliseconds in dark after a flash-excitation, is shown to decrease rapidly. A decrease in the fluorescence intensity could be explained

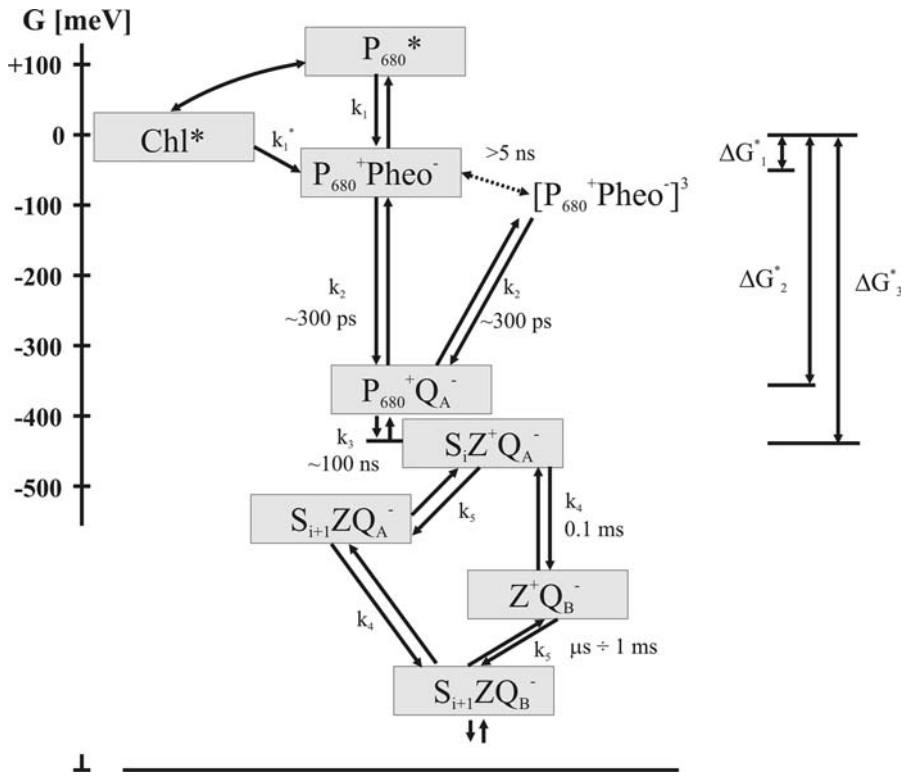

Fig. 1 Energy level diagram for the Photosystem (PS) II-states participating in Delayed Fluorescence (DF) generation. ΔG values indicate differences in Gibbs free energy between the states participating in ns to ms DF generation. k_1^* is the rate constant of excited antenna state decay by primary charge separation in PS II; k_1 is the rate constant of primary charge separation in excited PS II reaction center chlorophyll; k_2 is electron transfer (ET) from Pheo to Q_A; k_3 is ET from Z to P_{680}^+; $P_{680}^+Pheo^-$ is the singlet and $[P_{680}^+Pheo^-]^3$ is the triplet state of PS II radical pair, respectively. The rate constants k_3 and k_5 represent ET in the donor and k_4—in the acceptor side of PS II. For selected rate constants, the approximate values of the corresponding time constants, i.e., the reciprocal rate constants are given. The G value of the Chl_{200}^* state is chosen to be zero. Modified after Grabolle and Dau (2005)

by 1) reoxidation of the reduced acceptor (Q_A^-) and the transition from one radical-pair state $Z^+Q_A^-$, to another radical-pair state $Z^+Q_B^-$ (with rate constant k_4) or by 2) the reduction of Z^+ and the transition (with rate constant k_5) from state $S_{i-1}Z^+Q_B^-$ to state $S_iZQ_B^-$. A third reaction that decreases the concentration of the separated charge couples is charge recombination between Z^+ and Q_A^-. The kinetics of the DF decay depends on the rates of the three reactions. If the direct redox reactions are broken by physical or chemical treatments, the concentration of charge-separated states will decrease with time because of recombination and emission. In this case, the DF decay can be described by first-order kinetics (Tyystjarvi and Vass 2004):

$$\begin{aligned} L(t) &= c\varphi_f k_r^* [Z^+Q_A^-](t) \\ &= c\varphi_f k_{r0}^* [Z^+Q_A^-](0) \times \exp\left(\Delta G_3^*/k_BT\right) \\ &\quad \times \exp\left[-c\varphi_f k_{r0}^* \cdot t \cdot \exp\left(\Delta G_3^*/k_BT\right)\right], \end{aligned} \tag{4}$$

where, $[Z^+Q_A^-](t)$ is the temporary concentration of reaction centers (RC) in the $Z^+Q_A^-$ state during the measurement period; $[Z^+Q_A^-](0)$ is its concentration at the beginning of measurement; $k_r^*$is the rate constant of repopulation of Chl^* after recombination of radical-pair $Z^+Q_A^-$; k_{r0}^* is the temperature-independent part of the recombination rate constant (frequency factor) and can be determined experimentally.

In biological systems, where the separated charges are stabilized by the redox reactions on the acceptor and donor sides, the kinetics of the DF dark relaxation is significantly more complex and includes many kinetic components.

Measurement of delayed fluorescence

There are two principal approaches to the measurement of DF. One of these is to measure the curve of the DF dark relaxation after excitation with a saturating flash of light (of ns duration), after which different kinetic components of the curve and their relationship with the processes of charge transfer in the electron-transfer chain of the chloroplasts are evaluated. In the second approach, the induction curve (IC) of the DF is recorded during the transition of dark-adapted samples to light-adapted state.

Delayed fluorescence dark relaxation curves

There are two approaches to the analysis of the dark relaxation of DF—measurement after illumination of a dark-adapted sample by a single pulse (usually by a laser), or measurement after continuous illumination. The two approaches give different results. In both cases, the low intensity of the DF requires that several curves be averaged in order to improve the signal-to-noise ratio (Lavorel et al. 1986). However, with advancement in instrumentation (gated photomultipliers, better amplifiers, etc.) used for DF measurement, it is now possible to get excellent signal-to-noise ratio even from a single DF decay. To cover wider time range, Holgar Dau and co-workers (Grabolle and Dau 2005; Buchta et al. 2007) split the signal from the photomultiplier and then applied different amplifications to the two channels. Then, the signals of the two channels were analyzed at different time intervals.

In general, it is a good practice to start measuring DF as soon as possible after the excitation light is switched off; further, it is important to avoid both the scattered light and the prompt fluorescence, especially during the first nanoseconds. The scattered light is easily removed by placing complementary filters in front of the detector, since the wavelength of fluorescence is longer than the one of the scattered light (the exciting light). To avoid the detector form measuring prompt fluorescence, the detection system is turned off during the first nanoseconds after the illumination of the sample. This is achieved either electronically (by using gated photomultipliers) or mechanically by placing a shutter in front of the multiplier. Both methods have problems: the electronic method often gives artefact signals (Grabolle and Dau 2005), and the mechanical shutters are, in most cases, too slow.

Even in the relatively short period of time in which the DF relaxation curve is recorded, several kinetic components have been resolved (Lavorel et al. 1986).

Delayed fluorescence induction curves

When a dark-adapted sample is exposed to continuous light, we can measure DF induction curve, just as we measure PF induction curve. Both measurements reflect changes in the photosynthesis machinery during dark-to-light adaptation. For DF induction, however, it is necessary to use alternating light and dark cycles (Fig. 2). During the light period, prompt fluorescence can be measured, but during the dark period, DF is measured with one caveat: right after light period, the DF detector is kept off for a short period to avoid measuring PF decay. For millisecond DF measurements, the light and dark periods vary from several hundred μs to several ms. Usually, a phosphoroscope is used, the main part of which is a mechanical modulator providing the light-dark cycles; continuous light is usually chopped by a rotating disk. The duration of the light and dark periods (when DF is measured) is controlled by the rate of rotation of the disk and the size and position of the openings (Radenovic et al. 1994).

The DF signal, measured in each dark interval, is a polyphasic decrease of the DF intensity. Frequently, a distinct time period is chosen, and its averaged DF intensity is then analyzed (Zaharieva and Goltsev 2003). This averaging

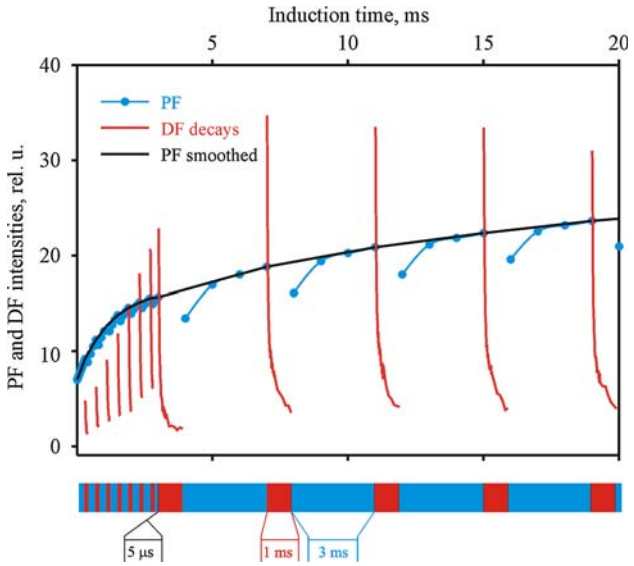

Fig. 2 Schematic representation of the timing protocol of an electronic-based non-phosphoroscopic M-PEA (Hansatech Instruments, King's Lynn, UK) for simultaneous recording of prompt (*blue points*) and delayed chlorophyll fluorescence (*red lines*). Black line represents the prompt fluorescence (PF) transient drawn through PF points recorded before the dark periods (PF smoothed). *Bottom bar*: Time scheme of alteration of actinic light and dark periods. During the periods marked with *blue color* the actinic light is on and chlorophyll fluorescence is measured. With *red color*, we have marked the time intervals when the light is off and delayed fluorescence is recorded. The sensor begins DF measurement 5 µs after the actinic illumination is switched off (marked with thin black line between blue and red regions). One measuring cycle includes light and dark periods in the ratio 3:1. The cycle duration increases during the induction in sequence: 400 µs, 4, 10, 100 ms, 1 and 10 s. The DF measurement period increases from 100 µs to maximal value of 240 ms

results in a single point of the IC. Using several time points, a complete IC can be built. Alternatively, the full DF decay is recorded after each illumination, and each curve is analyzed by multi-exponential simulations (Fig. 2).

An obvious advantage of the type of measurement, described above, is that during the illumination periods, PF can also be recorded, and thus one can simultaneously obtain and compare both PF and DF induction curves. The variable part of PF intensity decreases slightly (a few percent change) after every dark period as a result of Q_A^- dark reoxidation. During the same dark period, DF value drops drastically ($\sim$10 times or more). This suggests that the Q_A^- dark reoxidation does not determine DF µs dark decay. We speculate that DF µs dark relaxation is predominantly the result of redox reactions on the donor side of PS II.

One of the problems of the phosphoroscopic method is that the measured light intensity is a complex function of the history of sample illumination in the previous measurement cycles. This means that in each cycle there will be very slow components that have not yet decayed but that were excited during previous illumination cycles. These components may be low in amplitude, but they add up and at later stages of the induction period, they might significantly modify the recorded DF (Lavorel et al. 1986). A way to avoid this pile-up of the slow components was proposed by Schreiber and Schliwa (1987), who subtracted the signal at the end of a measuring cycle from the DF intensity in the next cycle.

Review of delayed fluorescence decay

The DF decay up to ms time domain is a result of the equilibrium reactions in PS II (Radenovic et al. 1994; Goltsev et al. 2005) discussed later in this paper.

Photosystem II has a shallow trap in P_{680} since excited energy states of the antenna chlorophyll *a* molecules are at similar energy levels; further, there is a fast equilibrium between the excited state P_{680}^* and the ion-radical pair $P_{680}^{\bullet+}Pheo^{\bullet-}$ (reactions 3 and −3 in Fig. 3) (Schatz et al. 1988). Delayed fluorescence that originates from the recombination of $P_{680}^+Pheo^-$ with a lifetime of 2–4 ns (Shuvalov and Klimov 1976; Jursinic 1986), can not be distinguished from PF (Schatz et al. 1988; Christen et al. 2000). This DF component can be measured (Shuvalov and Klimov 1976) only when the rest of the states that are in equilibrium with $P_{680}^+Pheo^-$ have been eliminated through a suitable chemical treatment. At 77 K, the excited state of P_{680}^* is, however, not shared with antenna chlorophyll *a* and DF originates directly from the PS II reaction center (Mimuro et al. 2007) and decays with lifetime in the range of 15–25 ns.

The decay of DF in the ns time range reflects the kinetics of reduction of $P_{680}^{\bullet+}$ by Z (reaction 5 in Fig. 3) (Christen et al. 2000). For thylakoid membranes, this decay is described by three exponentials with lifetimes of about 50 ns, 300 ns and 1.5 µs. The last two DF kinetic components oscillate, with a period of 4, with the number of the saturating pulses; these results show that they are related to the processes on the PS II electron donor side. In isolated preparations of light-harvesting complex of PS II, there is DF, and it decays with lifetimes of 65 and 800 ns, which suggests that these DF components are related to the formation of small quantities of radical pairs $Chl^{\bullet+}Chl^{\bullet-}$ even in the antenna complexes and to their radiative recombination (Christen et al. 2000).

When Q_A is reduced, three light emission components are observed, with lifetimes of 1 µs (Van Best and Duysens 1977), 5–10 µs, and 35–40 µs (Jursinic and Govindjee 1982); these are suggested to arise as a result of charge recombination in the $ZP_{680}^+PheoQ_A^-$ state (Sonneveld

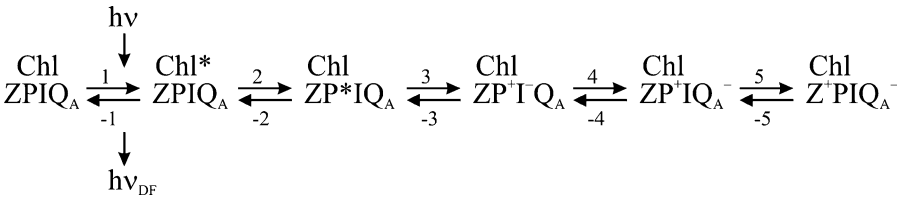

Fig. 3 Scheme of reactions in Photosystem II leading to Delayed Fluorescence (DF) emission. Here, I stands for an intermediate that is equivalent to pheophytin

et al. 1980b). It is possible that this type of light emission is a result of the formation of triplet states $[P_{680}{}^{+}Pheo^{-}]^{T}$, which is accelerated by the presence of $Q_A{}^{-}$, and of their reversal to the $[P_{680}{}^{+}Pheo^{-}]^{S}$ states (Jursinic and Govindjee 1982; Jursinic 1986). However, triplet exciton model for DF has not been supported (Stacy et al. 1971). It has been suggested that the lifetime of the 5–10 μs component also depends on the rate of electron transfer from Z to $P_{680}{}^{+}$ (Lavorel 1973).

Ten μs after photon absorption, about 80% of all PS II are in $Z^{+}Q_A{}^{-}$ state (Jeans et al. 2002). At this time, an equilibrium between the populations of the excited antenna state, $Chl_{200}{}^{*}$, and of the $Z^{+}Q_A{}^{-}$ state is reached (Grabolle and Dau 2005). From this moment up to the ms time range, most of DF is a result of charge recombination between Z^{+} and $Q_A{}^{-}$ (Van Gorkom and Donze 1973; Jursinic 1986). In the μs time domain, the DF decays are dominated by processes on the (electron) donor side of PS II. In dark-adapted PS II membrane particles, the DF dark relaxations show strong dependence on the number of the saturating laser pulses (Grabolle and Dau 2005). The DF decays induced by the third flash are kinetically resolved in four components: three are in the μs time range with time constants of 14 μs, 65 μs, 203 μs, which reflect steps in the $S_3 \rightarrow S_4$ transition and one slow, ms component related to dioxygen formation during $S_4 \rightarrow S_0$ transition (Buchta et al. 2007).

The sub-ms (120–200 μs) component (Lavorel et al. 1982; Gekhman 1988) in the DF decay curve is associated with the normal functioning of the PS II reaction center (Gaevsky and Morgun 1993). Goltsev (2005) suggested that it is due to leakage type luminescence generated from the reaction centers in the $Z^{+}P_{680}Q_A{}^{-}Q_B$ state, and the deactivation of these states is a result of the forward reaction of re-oxidation of $Q_A{}^{-}$ by Q_B, which occurs with a rate constant of 2500–5000 s^{-1} (Lazar 1999).

The first real ms component of the DF decay curve in stationary "light" conditions (after long light-dark cycles) has a lifetime of about 1 ms (Gaevsky and Morgun 1993) to 2–3.5 ms (Zaharieva and Goltsev 2003; Goltsev et al. 2005). Its amplitude is an order of magnitude lower than that of the μs component in isolated pea chloroplasts, but is higher in whole leaves (Gekhman 1988). With laser pulse illumination this DF component has a time constant of 1.2–2 ms and is clearly S-state dependent (Goltsev et al. 1980; Buchta et al. 2007). When DF is measured with the phosphoroscope method, after a few light cycles, the S-state distribution is changed. The DF quantum yield of S_3-state exceeds by an order of magnitude of that of the S_2-state, and by 3 orders of magnitude of that of the S_1-state (Buchta et al. 2007). When there is a random distribution of different S-states, the DF is emitted mainly by PS II in the S_3-state. DF decay in the ms time domain is a result of "leakage", both on the donor and the acceptor sides. At the donor side, DF lifetime may reflect mono-exponential dioxygen formation and disappearance of S_3-states. At the acceptor side, DF dark relaxation is related to the reoxidation of $Q_A{}^{-}$ by the electron transport chain with fully reduced Q_B ($Q_B{}^{=}$). In a chloroplast suspension with an exogenous acceptor (especially under uncoupling conditions) and in whole leaves, the lifetime of the ms component probably reflects, besides reactions in oxygen evolving complex, the kinetics of oxidation of reduced PS II quinone acceptors by the plastoquinone (PQ) pool (Gaevsky and Morgun 1993; Goltsev et al. 2005). The following equation shows these acceptor side reactions:

$$S_3Z^{+}P_{680}Q_A{}^{-}Q_B{}^{=}+PQ+2H^{+} \rightarrow S_3Z^{+}P_{680}Q_A{}^{-}Q_B{}^{-}+PQH_2$$

The acceleration of the electron transfer is expected to be accompanied by a decrease of the lifetime of the ms component.

The amplitude of the ms component depends on the difference in the potentials on the two sides of the membrane (Fleishman 1971; Venediktov et al. 1980). Interestingly, it decreases under both phosphorylating conditions and when uncouplers of phosphorylation are present (Wraight and Crofts 1971). A major explanation is that transmembrane electric field facilitates the electron transfer from $Q_A{}^{-}$ to Pheo, and then to $P_{680}{}^{+}$, reducing the activation free energy of the charge recombination. In thylakoid suspensions, the intensity of ms DF increases exponentially with increasing transmembrane diffusion electric gradient (see Jursinic et al. 1978; Venediktov et al. 1980).

Delayed fluorescence that decays with lifetimes of about several tens of seconds has been investigated by many authors (Rutherford et al. 1984; Rutherford and Inoue 1984; Hideg et al. 1991; Katsumata et al. 2008; Berden-Zrimec et al. 2008). The slow DF components (with lifetimes of several seconds) relate to the recombination of the S_2 and S_3 states of the oxygen evolving complex with $Q_A{}^{-}$ and $Q_B{}^{-}$ (Joliot et al. 1971; Lavorel 1975). This component has been identified to originate from recombination occurring in RCs

in the $S_2Q_B^-$ or $S_3Q_B^-$ state. Its lifetime correlates with the time of re-oxidation of Q_B^-, which is about 22 s (Robinson and Crofts 1983).

The slow decay of luminescence in the second time range is well described by a second-order kinetics. The experimental decay curve is fitted well with a first or a second-order hyperbola (Tyystjarvi and Vass 2004 and references therein).

When the photosynthetic sample is illuminated by infrared light, the relaxation curve has a maximum at 30–40 s after the light is switched off (Katsumata et al. 2008 and references therein). The complex kinetics of the dark relaxation and emission spectra of the slow components indicate that both photosystems I and II take part in the generation of excited states of PS II antennae chlorophylls and by this way—in the emission of this type of luminescence (Schmidt and Senger 1987).

Induction curve

During the illumination of dark-adapted leaves, the DF intensity, like the PF intensity, undergoes a series of complex induction transitions that reflect the function of the photosynthetic electron transport chain (Malkin 1979). The PF induction curves have been extensively studied and used as a tool in photosynthesis research and plant physiology (Dau and Hansen 1989; Dau 1994; Lazar 1999; Schreiber et al. 2000; Tsimilli-Michael and Strasser 2008).

The DF induction curve reflects processes that occur in photosynthesizing samples when they are exposed to light after a period of dark adaptation. The best resolution of the IC maxima can be obtained after a dark adaptation period of 5–15 min. After the induction period, which lasts about 2–3 min at room temperature and saturating light intensity, a stationary level of DF is reached (Veselovskii and Veselova 1990; Radenovic et al. 1994).

We know much less about the DF induction curve than about the PF transients. The DF induction curve, unlike that of PF, has several maxima and minima. Their number and amplitude, however, depend on the recording period, or in other words, on the kinetic components of DF being measured (Zaharieva and Goltsev 2003). Moreover, the amplitudes and lifetimes of the ms DF during the induction period vary significantly (Goltsev et al. 2005). There is no consensus nomenclature of the maxima that are observed in the DF induction curve, and no consensus about the number and interpretation of these maxima. Goltsev and coworkers (Goltsev and Yordanov 1997; Zaharieva and Goltsev 2003; Goltsev et al. 2005) suggest that the maxima (denoted by I) and minima (labelled as D) should be numbered in sequence according to their position in the IC (I_1, D_1, I_2, D_2 etc.).

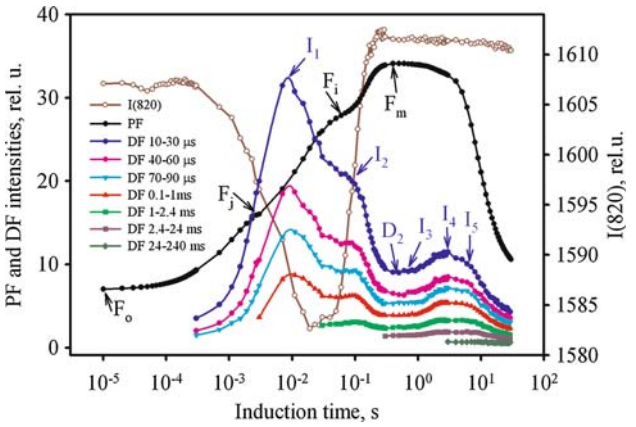

Fig. 4 Simultaneous measurements of light absorption measured as relative changes of photocurrent at the upper side of the leaf at 820 nm (I(820), brown line, right axis), Prompt Fluorescence (PF, blue line, left axis) and Delayed Fluorescence (DF, left axis), recorded from 10 µs to 100 s. The induction curves were recorded in dark adapted *Camellia* leaves from 10 µs to 20 s of illumination. Actinic light intensity was 5000 µmol (photons) m^{-2} s^{-1}. These transients were measured at the Hansatech Instruments Company (UK) during 2008 and 2009 with their new instrument labeled M-PEA

The changes in DF intensity during the induction period are correlated with different processes that include the build-up of the electrical and proton transmembrane gradient (Wraight and Crofts 1971), and depend on the availability of electron acceptors (Ruby 1976) and donors (Mar et al. 1975), and on the state of the oxygen-evolving complex (Zankel 1971; Van Gorkom and Donze 1973).

Two phases can be observed in the DF induction curve (Fig. 4): a fast and a slow one, lasting 200 ms and several minutes, respectively (Itoh et al. 1971; Itoh and Murata 1973; Malkin and Barber 1978). The fast phase includes two maxima I_1 and I_2, after which DF drops to a minimum labelled as D_2 (Goltsev and Yordanov 1997; Goltsev et al. 2003). After a small step, labelled as I_3, the slow phase begins. During this phase DF rises to a maximum I_4 and then, through several transient maxima (I_5 and I_6), DF intensity decreases to a stationary level S (Itoh and Murata 1973; Goltsev et al. 2003).

The fast phase of the DF induction curve occurs during the increase of the PF intensity from F_o to F_p (see Papageorgiou and Govindjee 2004).Two maxima can be seen in this phase—I_1 and I_2. The first one, I_1, coincides with the increase of PF from F_o (the "O" level) to F_i (the I level). At this time, the transient decrease of I(820) (Fig. 4) shows that reduced plastocyanin and P_{700}^+ must have accumulated, probably due to the lack of donors for PS I (lack of reduced plastoquinone) (Schansker et al. 2003). This results in the accumulation of a transmembrane electrical gradient (Satoh and Katoh 1983). The appearance of I_1, like the transition from F_j (J level) to F_i (I level), can be related to two phenomena:

(1) photochemical—accumulation of certain light-emitting states of the PS II RC, and (2) non-photochemical—increase of the DF due to the electrical gradient formed by PS I when P_{700} is oxidized (Pospisil and Dau 2002). The I_2 maximum (usually only a shoulder) is probably related to the prolonged reopening of PS II RCs by the electron transfer from the reduced Q_B to PQ before the full reduction of the PQ pool (I_2–D_2 transition). The relative size of this maximum increases with the decrease of the size of the PS II antenna and when the measuring temperature is increased (Zaharieva et al. 2001).

After about 0.5 s of illumination, because of the reduction of the plastoquinone pool, there is a decrease of the outflow of electrons from Q_A^-, and the RCs shift to a "closed" state $ZP_{680}Q_A^-Q_B^=$ (phase D_2) (Gaevsky and Morgun 1993; Zaharieva and Goltsev 2003; Goltsev et al. 2005). Under these conditions, the amplitude of the 160-μs component decreases, and the lifetime of the ms component increases (Zaharieva and Goltsev 2003). In the presence of ferricyanide, an artificial electron acceptor, and uncouplers of the phosphorylation, this increase of the lifetime is insignificant and no I_2–D_2 is observed. This indicates that the I_1–I_2–D phase correlates with the processes of reduction of the PQ pool, and the time when the D_2 minimum is reached, can be an indicator of the reducing activity of the PS II complex (Gekhman 1988).

The increase of DF to the next maximum, labelled as I_4, occurs in parallel with a slight decrease of the PF intensity and oxidation of P_{700} (Goltsev et al. 2005). The accumulation of P_{700}^+ suggests that at this time the light-induced activation of the ferredoxin:$NADP^+$-oxidoreductase takes place (Harbinson and Hedley 1993; Schansker et al. 2006), i.e., the linear electron transport is activated, and the transmembrane proton gradient starts to accumulate. The increase of the DF intensity in the slow phase (towards the I_4 maximum) is associated with the formation of the proton gradient (Wraight and Crofts 1971; Evans and Crofts 1973) that increases the rate constant of radiative recombination in the PS II RCs.

On the other hand, it can be assumed that the increase of luminescence during the D_2–I_4 phase is not directly connected with the transmembrane proton gradient formation, but instead to the neutralization of the negatively charged buffer groups that are present on the internal surface of the thylakoid membrane. During the DF increase to the I_4 maximum, an additional difference in the electric potentials appears that is caused by the accumulation of Mg^{2+} ions inside the thylakoids as a result of a H^+/Mg^{2+} exchange when the buffering capacity of the thylakoids is saturated (Gaevsky and Morgun 1993). Later, this potential decreases when Mg^{2+} is exported out of the thylakoids along the gradient of the electric potential. This process describes the decrease of DF in the phase I_4–I_6 and can be observed in chloroplast suspensions in non-phosphorylating conditions, when ΔpH produces only osmotic work (Grigoryev et al. 1982). During continuous illumination, ΔpH of the thylakoid membrane increases while the electrical component of the membrane potential decreases to a minimal value (Rottenberg 1977). Grigoryev et al. (1982) suggested that the decrease (I_4–I_6–S) of the induction curve partly reflects the changes in the transmembrane electrical gradient ΔΨ, which occurs as a result of the secondary ion transport (Rottenberg 1977).

The peak M_2 that appears late in the PF induction curve is usually associated with the activation of the dark reactions of the Calvin–Benson cycle (Ireland et al. 1984). After this maximum, the acceleration of the electron transport induces a decrease of the PF that coincides with the I_6 peak of the DF induction curve. The maximum I_6 can only be seen at a temperature 25°C or higher.

The amplitude of the I_4–I_6–S phase in the DF induction curve in whole leaves is much larger than the one seen in chloroplasts (Gaevsky and Morgun 1993). In chloroplasts suspended in a medium without an electron acceptor, this decrease is rarely observed (Itoh et al. 1971; Grigoryev et al. 1982). In intact leaves the cause of the DF decrease is the start of the CO_2 fixation cycle, the consumption of ATP (Pavlova et al. 1978) and the acceleration of the kinetics of millisecond DF dark decay (from 4 down to 2 ms, see Fig. 5).

Delayed fluorescence intensity can be integrated for different DF decay intervals and can be presented as different induction curves. Some experimental setups allow optional selections for one or more decay intervals (Fig. 4). The measurement of induction curves for the DF that decays in different intervals allows the representation of the DF intensity as a function not only of the induction time, but also of the interval of the measurement.

Figure 5 shows the change of the parameters (amplitude, *L*, and lifetime, τ), obtained after deconvolution of the DF decays, and recorded at different times during the induction period in intact leaves.

The changes in the time constants of the sub-ms and ms DF decays are modulated by photoinduced changes of the oxygen evolving complex (predominantly in S_3-state), the state of the PS II acceptors, and the energization of the thylakoid membrane and the temperature of the sample. The comparison of the rate constants of electron transfer reactions in the PS II acceptor side with the lifetimes of measured DF kinetic components shows which transitions between the particular states of the PS II reaction center determine DF decays (Zaharieva and Goltsev 2003).

The emission decaying in the sub-ms time domain is connected with the $Z^+P_{680}Q_A^-Q_B^-$state, and the one decaying in the ms time domain is connected with the $Z^+P_{680}Q_A^-Q_B^=$ state. The lifetimes (τ_1 and τ_2) are determined by the probabilities of forward and backward electron transport in the PSII states, described above. These

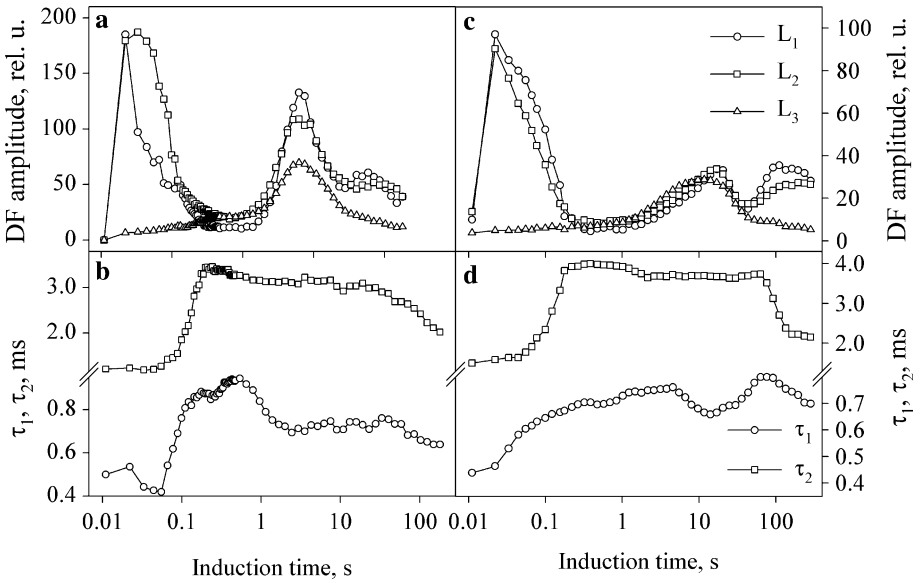

Fig. 5 Time course of dark relaxation parameters of delayed chlorophyll fluorescence in barley leaves (**a**, **b**) and leaf disks from *Arabidopsis thaliana* (**c**, **d**) during light to dark adaptation. Several parameters were calculated by fitting of the experimental DF decays using the following equation: $DF(t) = L_1 \times \exp(-t/\tau_1) + L_2 \times \exp(-t/\tau_2) + L_3$, where, L_1, L_2 and L_3 are the amplitudes of the kinetic components (**a**, **c**); τ_1 and τ_2 are lifetimes (**b**, **d**) (Goltsev et al. 2003)

assumptions are in agreement with experiments done with the modifiers of PS II electron transport (Goltsev et al. 1998) or of thylakoid membrane fluidity (Zaharieva et al. 1998), as well as with the interpretation of temperature dependences of DF decay parameters (Zaharieva et al. 2001). The ms time constant increases approximately twofold during the first second of illumination (Fig. 5). This increase, observed at the beginning of the induction period, is presumably a result of the photoinduced PQ pool reduction (Goltsev et al. 1998): when the PQ pool is reduced, one of the ways for disappearance of the $Z^+P_{680}Q_A^-Q_B^=$ state (namely, dissociation of the PQ molecule from the Q_B binding site) becomes blocked. After the first few seconds of illumination, the lifetime of this component only slightly decreases. Therefore we can conclude that after PS I activation (photoactivation of the ferredoxin-$NADP^+$-reductase), the PQ pool is only partially reoxidized. The main part of the DF increase, in this time scale, is probably due to the photoinduced proton gradient but not to the reopening of PS II reaction centers (Goltsev et al. 2003).

The PQ pool maintains some level of reduction after PS I activation. Further, a sharp decrease in the ms lifetime can be observed that coincides with the activation of the Calvin–Benson cycle enzymes and reopening of the PS II reaction centers. It is evident that the PF decrease after the maximal level F_p (the P level) is due to non-photochemical quenching rather than PQ pool reoxidation. This is in agreement with the work of Briantais et al. (1980), who showed that P-to-S decay in PF is a result of energy-dependent quenching by photoinduced lumen acidification.

Relationship between prompt and delayed fluorescence

Information about the state of the photosynthetic machinery can be obtained from the shape of the DF induction curve and from the analysis of its correlation with the simultaneously recorded PF transient.

For a better understanding of the nature of the maxima that are seen in the DF induction curve, this curve is often compared to the PF transient placed on the same time scale (Govindjee and Papageorgiou 1971; Krause and Weis 1991; Malkin et al. 1994). The DF and PF induction curves reflect the same changes of the redox state of the PS II reaction center (Itoh 1980). The comparative analysis of PF and DF allows the investigation of the routes of utilization of the absorbed energy and photosynthetic activity. A close correlation between DF and PF was shown in leaves with inactive PS II (plants grown under the regime of 1 ms flash given after every 15 min of darkness). During continuous illumination of such leaves, the rise of the photosynthetic activity and the oxygen evolution rate paralleled the appearance of DF induction and variable fluorescence transients (Srivastava et al. 1999). The simultaneous study of PF and DF was carried out mainly with isolated chloroplasts, which do not display the complex induction behaviour that is characteristic of intact leaves. The ms DF with a lifetime of 2–3 ms does not correlate with the changes of PF, while for the longer DF components, such a correlation is observed (Clayton 1969; Malkin and Barber 1978).

The problem of comparison of the DF with PF transient is that the ms DF usually includes overlapping fast and slow kinetic components, which behave in a different way during the induction period (Mar et al. 1975).

In summary, the ms DF induction reflects the changes in the redox level of P_{680} and Q_A at low and room temperatures (Itoh 1980). The induction curves of the ms DF and PF transients have an anti-parallel relation (except for the time immediately after the start of illumination) at different experimental conditions—the ms DF is inversely proportional to the Q_A^- concentration (Itoh 1980). After continuous illumination, the S-states of the oxygen evolving complex are unsynchronized (approximately equally distributed) and the S-state transitions have presumably a small effect on the DF induction transitions, although the different S-states yield a different DF intensity at room temperature or at over −20°C (Velthuys and Amesz 1975).

The slow-decaying DF is observed only at room temperature and has an entirely different induction curve, which is parallel to the PF induction curve (Clayton 1969; Malkin and Barber 1978). The slow-decaying DF consists of components that are due to the reversal of the electron transfer at later stages of the electron transfer process (Itoh 1980). The parallel course of PF and the slow DF components is even more apparent in the presence of the uncoupler valinomycin (Satoh and Katoh 1983).

As the PF and DF are controlled by the same processes that take place during the transition from a dark-adapted to a light-adapted state, the relationship between them can be studied theoretically.

Analyzing the intensities of DF and PF recorded simultaneously using the phosphoroscopic method, Goltsev et al. (2003) related the ratio L/Fv to the ratio of the oxidized and reduced states of Q_A (B) and to the energization of the thylakoid membrane

$$L/Fv \sim [(1 - B)/B] \times \exp\left(-\frac{E_a - F\Delta\psi + 2.3RT\Delta pH}{kT}\right) \times \varphi F_o/\varphi F_m,$$

where, E_a is the activation energy of radiative recombination, $\Delta\psi$ is the membrane potential; φF_o and φF_m are the quantum yield of fluorescence from PS II with open and closed RC, respectively, k is the Boltzmann constant and the other symbols have their usual meaning.

A new term was introduced—luminescence potential (U_L), which is the sum of the potentials that lead to the formation of DF. If we assume U_L is equal to the logarithm of the ratio L/F_v, then U_L would be proportional to the sum of the potentials:

$$U_L = \ln(L/F_v) \sim E' + \Delta\mu H^+$$

The phase diagrams $V(U_L)$ and $L(U_L)$ presented in Fig. 6 show the changes of the variable chlorophyll fluorescence (V) and DF intensity (L) on the U_L. This figure illustrates the effect of photosynthetic parameters (redox potential of the PS II acceptors and the thylakoid membrane energization) on PF and DF.

We propose that in the linear regions, only one of the two components of U_L (redox term $E' = \ln((1-B)/B)$ or $\Delta\mu H^+$) changes the PF or DF, and the other component remains relatively constant. The slopes of the linear regions reflect the sensitivity of the PF or DF to the corresponding U_L component (Goltsev et al. 2003).

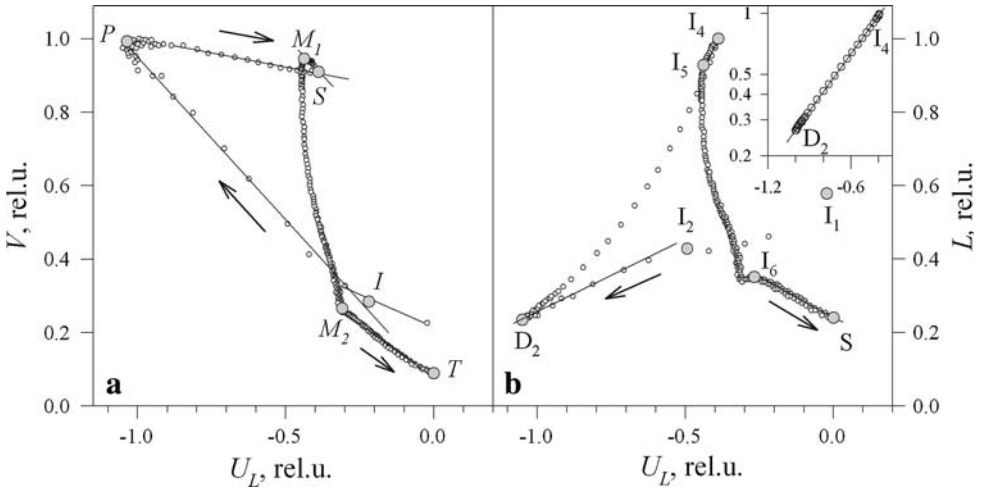

Fig. 6 The course of the changes of relative variable chlorophyll fluorescence, V (**a**) and delayed fluorescence, L (**b**) during dark to light transition as a function of luminescence potential ($U_L \sim E' + \Delta\mu H^+$) in barley leaves. Grey circles represent characteristic points of PF and DF induction curves, as in Fig. 4. All data are normalized to the corresponding maximal values. Experimental conditions were as in Fig. 1 in Goltsev et al. (2003). Inset: part of the experimental points from D_2 to I_4 are presented on a semi-logarithmic scale. The straight line shows linear regression of the experimental points. The figure is redrawn from Goltsev et al. (2003)

In this way, the I–P (F_i–F_p) section of the PF transient reflects the photo-induced reduction of the acceptor side of PS II, and the slope represents a proportionality coefficient between the amount of reduced acceptors of PS II and the increase of PF. The slope of the O–I (F_o–F_i) section is much smaller than the one of the I–P section and is close to the one of the P–S phase that is related to the energization of the thylakoid membrane.

Why do we measure DF?

Plants do not use all the quanta they absorb with 100% efficiency. Some of the absorbed energy is emitted as prompt or delayed chlorophyll fluorescence, or as heat. Each DF quantum has its pre-history—how many and which of the forward and backward reactions have lead to the formation of the excited state of the antenna chlorophylls. Each emitted quantum carries a bit of information about these reactions.

The delayed fluorescence emission of PS II is a useful tool to quantitatively study the light-induced electron transfer and related processes (e.g., proton movement) which are associated with a free-energy drop (Buchta et al. 2007). This method provides insights in the functionally important inner-protein proton movements, which are hardly detectable by conventional spectroscopic approaches (Buchta et al. 2007).

Based on DF, a rationale for the determination of the free energy differences between the excited-antenna state and the radical-pair state, reached at a given time after the flash, has been proposed (Grabolle and Dau 2005). Differences in energies of several PS II redox states can be calculated reasonably precisely. Based on the measured DF decays from oxygen-evolving PS II membrane particles, an attempt was made to obtain redox-potentials of the electron carriers involved (Grabolle and Dau 2005).

The ability of one single equipment to measure simultaneously light emission (both PF and DF) and absorption changes, for example at 820 nm (Srivastava et al. 1999) and at 320 nm (characterizing the redox states of P_{700} and Q_A) is valuable in obtaining information about the function and, to some extent, about the structure of the photosynthetic machinery in the same sample. Such instruments are beginning to be available now.

One of the major advantages of DF is the possibility to measure it in native samples. The high sensitivity of photosynthesis to environmental factors, and the sensitivity of DF to changes in the different photosynthetic processes have made DF a useful tool for testing the plant reactions under stress conditions (Bilger and Schreiber 1990; Gaevsky and Morgun 1993). Using a suitable experimental approach, DF has already been used for the analysis of the effects of chemical and physical factors: high and low temperatures (Itoh 1980; Goltsev et al. 1987; Yordanov et al. 1987; Bilger and Schreiber 1990), light stress (Valikhanov et al. 2002) and UV irradiation (Zhang et al. 2007a), drought and high salinity (Mladenova et al. 1998; Zhang and Xing 2008), herbicides (Lambrev and Goltsev 2001), heavy metals (Plekhanov and Chemeris 2003), harmful gases (Ellenson and Amundson 1982). The ms DF can also be used to monitor effects of viral and bacterial diseases on plants (Milanov et al. 1997; Christov et al. 2001), and even to a change in the physiological state of the plant cell during its ontogenetic development, greening and different hormonal levels (Srivastava et al. 1999; Zhang et al. 2007b; Yordanov et al. 2008).

The DF-imaging is highly useful for the visualization of the spatial differences in the physical state of leaves due to mosaic distribution of the viral and bacterial infections on them (Björn and Forsberg 1979; Ellenson and Amundson 1982).

In micro-algal populations, DF has been used as a measure of total photosynthetic activity (Monti et al. 2005), reflecting also the algal biomass (Yacobi et al. 1998; Katsumata et al. 2006). The algal cells are very sensitive to toxic compounds and the DF measurements of algal systems are used as ecotoxicological bioassays, which allow the rapid estimation of the effects of inhibitors of algal growth (Katsumata et al. 2006; Berden-Zrimec et al. 2008).

Prompt fluorescence has been used as a tool to obtain quantitative information about the photosynthetic process in plants, algae and cyanobacteria (Govindjee et al. 1986; Papageorgiou and Govindjee 2004). For example, Reto Strasser and co-workers (Strasser et al. 1995; Tsimilli-Michael and Strasser 2008) have quantitatively related induction transients of PF with the activities of photosystems I and II and with the electron transfer between them. Regardless of the fact that the PF and DF are emitted by the same population of antenna chlorophylls of PS II, they carry different, complementary information about the quantitative characteristics of the photosynthetic process. At the present time, equipment for the simultaneous measurement of induction transients of PF, DF, decay curves of DF and changes in light absorption at 820 nm (P_{700}^+) with μs time resolution, is available (see Figs. 2 and 4). For the processing and evaluation of the large information that is obtained by such an equipment, mathematical models must be evolved to describe the photosynthetic reactions and their relation to the fluorescence characteristics. Such models describe the induction transients of PF and DF (Goltsev and Yordanov 1997; Markovic et al. 2001; Kalauzi et al. 2006), and the curves of DF decay (Goltsev et al. 2004; Li et al. 2007; Guo and Tan 2009). When the models are fitted to the experimental curves, we expect to obtain the values of rate constants of different photosynthetic

reactions. The combination of the modelling with the design of artificial neuron networks for specific global physiological properties of the plant (Chernev et al. 2006) would further increase the applicability of the method of delayed chlorophyll fluorescence in plant biology and agriculture.

Acknowledgements We thank the Bulgarian National Science Fund, Project No. DO 02-137/15.12.2008 for the financial support. R.J.S acknowledges support by the Swiss National Science Foundation, Project Nr: 200021-116765. We thank Detelin Stefanov for critical reading of the manuscript. The paper was written while I.Z. was working at the Free University in Berlin on an Alexander von Humboldt fellowship. This review was edited by Govindjee. We thank him for many suggestions that have improved this review.

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