Delayed fluorescence emission as a probe for the response of photosynthetic organisms to high temperature exposure: a comparative study

Abdallah Oukarroum ^{a, b, *}, Mohamed El Gharous ^a, Vasilij Goltsev ^c, Reto J. Strasser ^b

^a Mohammed VI Polytechnic University, School of Science of Agriculture, Fertilizing and Environment, Morocco

^b Bioenergetics and Microbiology laboratory, University of Geneva, CH-1254 Jussy-Geneva, Switzerland

^c Department of Biophysics and Radiobiology, Faculty of Biology, St. Kliment Ohridski University of Sofia, Sofia, Bulgaria

*Corresponding author: Dr A. Oukarroum

Email address: Oukarroum.abdallah@uqam.ca

Tel.: +1 514 987 3000

Fax: +1 514 987 4054

ABSTRACT

The effects of a short-term exposure (40 s) to 50°C on the prompt fluorescence (PF), delayed fluorescence (DF) and 820 nm reflection (MR) signals in three different photosynthetic organisms: *Pisum sativum* (angiosperm plant), *Ginkgo biloba* (gymnosperm tree) and thalli of *Parmelina tiliacea* (Hoffm.) Ach (lichen) were investigated. The aim of this study was to understand the relationship between prompt PF, DF and MR when the oxygen evolving complex (OEC) is destroyed. The signals of PF, DF and MR were measured simultaneously in plants *in vivo* to probe heat-induced damages in the photosynthetic electron transfer. PF is characterized by a polyphasic fluorescence rise OJPSMT and DF induction curve has several maxima (I_n) and numbered in sequence according to their appearance during illumination. In control condition (25°C) leaves of pea plants yield a typical OJIP rise with a flat P-step (0.2–2 s) and in *Ginkgo biloba* and *Parmelina tiliacea* thalli, the P-level was split into two peaks, the first peak P (0.2 s) is followed by a decrease of the fluorescence intensity to a dip (S) and a subsequent rise to a second peak (M). An antiparallel relation between J-I-P-S and DF curves was also observed. Methyl viologen (MV) reduced the amplitude of the I-P phase of the PF curve and this change was accompanied by a disappearance of the PSM transient. In the presence of MV, the DF intensity remained higher; and the I_4 peak disappeared. We suggested that P-S-M rise from PF and I_4 peak might be related to an acceleration of intersystem electron transport. After 50°C incubation, significant changes appeared in the PF, DF and MR transients and that were dependent on the photosynthetic sample. Heat treatment provoked a down regulation of electrons donation by the OEC in the three plant species and the re-reduction of $P700^+$ and PC^+ started earlier and began

already after 10 ms of illumination. Inhibition of the donation of electrons by OEC after 50°C incubation indicates that DF induction depends on the concentrations of Q_A^- and $P680^+$ and the proton gradient.

Keywords: Heat treatment; Oxygen evolving complex; Prompt fluorescence; Delayed fluorescence; 820-nm light reflection

1. Introduction

Prompt fluorescence (PF) emitted by photosynthetic organisms on a dark-to-light transition is characterized by a polyphasic chlorophyll (Chl) *a* fluorescence OJIP [1], [2], [3] and [4]. The fluorescence intensity increases quickly from a minimum fluorescence intensity (F_o) to a maximum fluorescence intensity (F_M) and with two intermediate steps labelled as J (F_J) and I (F_I). The OJIP transient can also be divided to two rise phases: the photochemical phase O-J and the thermal phase J-I-P [1], [5], [6] and [7]. This OJIP part was followed by a slower decrease towards a ‘terminal’ steady state level F_T [8]. The characteristics of the slow fluorescence decline following the P step differs between species. In angiosperm species the P-step is broad, whereas in other organisms the fluorescence intensity starts to decrease almost immediately after P is reached. In those organisms a second peak M is observed after a few seconds of illumination: e.g. in diatoms [9], foraminifers [10], *Pinus brutia* needles [11] and lichens [12] and [13]. In those cases we speak of a PSMT transient with S assigned to the minimum between the peaks P and M [14].

Recently, a technique was developed for studying the energetic behaviour of the stress-induced damage to the photosynthetic apparatus based on simultaneous measurements of the kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and the modulated 820 nm signal (measured in reflection mode) (MR). PF is emitted by a photosynthetic sample as long as it is illuminated, whereas DF can only be detected in the subsequent darkness. Like variable fluorescence, DF is mainly emitted by PSII [15] and reflects the occurrence of recombination reactions in photosystem II (PSII) [16], [17], [18] and [19]. Kinetic changes at 820 nm reflect changes in the redox states of the primary electron

donors of photosystem I (P700) and plastocyanin (PC) with a small contribution of ferredoxin [20] and [21].

The shapes of the PF, DF and MR transients depend on the photosynthetic sample and its physiological state [13], [22], [23], [24], [25], [26], [27], [28] and [29]. The PF transient (OJIP) depends on the redox state of the PSII reaction centers (RC) and reflects the subsequent reduction of the acceptor side of PSII, the PQ pool and the electron acceptors around PSI [21] and [30]. The DF induction curve depends on the redox state of the electron transport, the associated redox state of Q_A in darkness following illumination and the redox state of the donor side of PSII, as induced by the preceding illumination [31] as well as on the backward electron transfer rates [15] and [32]. In a time range from several microseconds to milliseconds of DF decay the emission has been thought to reflect the recombination in the dark between the reduced primary electron acceptor Q_A^- and the oxidised primary donor ($P680^+$) of PSII [16]. The DF induction curve has several maxima (denoted by I and numbered in sequence according to their position in the DF induction curve). Their number and amplitude, depend on the kinetic components of DF that were induced by the preceding illumination [22], [31], [32] and [33]. Two DF maxima (I_1 and I_2) are observed concomitant with the OJIP rise. Goltsev et al. [33] proposed that the I_1 peak is the result of the induction of a transmembrane electrical gradient and of the accumulation of RCs with semi-reduced Q_B ($Z^+P680Q_AQ_B^-$). Further, Schansker et al. [34] concluded based on the modeling work of Lazár [35] and the work of Grabolle and Dau [36] that the I_1 peak was mainly a reflection of a transient accumulation of relatively high population of $P680^+$ that reached after illumination of open PSII reaction centers in the state S_3P680Q_A . Lowering the effective light intensity shifts the I_1 peak to longer

times and will also reduce the amplitude of the peak, because the induction of the S_3 state in all the reaction centers together will happen over a longer time interval [34]. In this context it is important to emphasise, that the contradiction between the measurements of Schansker et al. [34] on the one hand and those of [23] on the other with respect to the peak position of I_1 that Stirbet and Govindjee [6] noted does not exist, but is due to differences in the protocols used. The peak position of I_2 in DF is associated with an increase of the population of PSII in the open state as a result of acceleration of PQ pool reoxidation by oxidized $P700^+$, that reopen PSII reaction centers [37].

The elevated temperature has multiple effects on the photosynthetic reactions. It was shown earlier that heat treatment induces a release of the extrinsic proteins, especially psbO [38], and [39], followed by a superreduction of the Mn-cluster by reductants like ascorbate in the lumen [40] leading to a release of at least two of the Mn-ions [39]. This process causes an irreversible inactivation of the oxygen-evolving complex (OEC) [41] and [42]. After 50°C incubation, a significant change appeared in the PF, DF and MR curves and the K step appeared in OJIP transient [24]. This K step is explained by the loss of the electron donation capacity of the donor side of PSII associated with the destruction of the Mn-cluster [43].

In this study, the effects of a short-term exposure (40 s) to 50°C on the PF, DF and 820 nm reflection (MR) signals in three different photosynthetic organisms: *Pisum sativum* (angiosperm plant), *Ginkgo biloba* (gymnosperm tree) and thalli of *Parmelina tiliacea* (Hoffm.) Ach (lichen) were investigated. In a previous study, Oukarroum et al. [13] reported that the mechanisms that increase the tolerance of higher plants to heat stress are absent or less well developed in lichen and it is important to evaluate heat response in

different plant species. Our hypothesis is that when the OEC is destroyed at 50°C in the three photosynthetic organisms; the properties of their electron transport chain and the relationship between DF, PF and MR changed. Also, we expect to obtain complementary information for the whole photosynthetic electron transport that increases the applicability and comprehension of this method in plant physiology.

2. Material and methods

2.1. Plant materials

Pisum sativum, *Ginkgo biloba* and thalli of *Parmelina tiliacea* (Hoffm.) Ach. were used in this study. The seeds of pea (*Pisum sativum* L. cv. Ambassador) were allowed to germinate for 48 h on a sheet of Whatman filter paper placed in Petri dishes. The germinated seeds were transplanted into plastic pots (4 l) containing commercial peat soil with three plants per pot and in five replicates. In the greenhouse, the plants were grown under controlled conditions with a day/night air temperature of 25/18° C, under long-day conditions (16 h light, 8 h dark) and by giving additional light if needed (OSRAM HQIT 400W lamps). Mature leaves of 2–3-week-old *Pisum sativum* plants were used. Healthy thalli of *Parmelina tiliacea* (Hoffm.) Ach. were collected from the south sides of oak trunks, around the laboratory in Lullier-Jussy, Geneva (Switzerland). The collected lichen thalli were first, air-dried for 48 h at room temperature under low light conditions ($\sim 12 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Leaves of *Ginkgo biloba* were sampled from branches collected from trees near of the laboratory in Lullier-Jussy, Geneva (Switzerland). Healthy leaves of a similar age were sampled from each branch.

2.2. The Multifunctional Plant Efficiency Analyser M-PEA

In the M-PEA instrument emitter wavelength ranges are: (a) 627 ± 10 nm, for the actinic light LED; (b) 820 ± 25 nm, for the modulated light LED, and (c) 735 ± 15 nm, for the far-red light LED; the latter uses a RG9 long pass filter to remove any visible light component [23], [24], [25], [29–33], and [40–44]. High quality optical band pass filters were used for the protection of the detectors of prompt and delayed fluorescence (730 ± 15 nm) and modulated reflection (820 ± 20 nm). The light of the actinic light LED is focused on the sample surface to provide homogeneous illumination with an intensity of $5000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ [45]. During the measuring cycle, the PF is measured when the actinic light is on and DF is recorded when the light is off. The calculated ratio MR/MR_0 (MR_0 is the value at the onset of the actinic) is complementary to the fraction $(I_{\text{abs}}/I_{\text{inc}})_{820 \text{ nm}}$ of incident light flux (I_{inc}) that is absorbed (I_{abs}) by the sample (at 820 nm). The first reliable MR time point was at 0.3 ms.

2.3. Temperature treatment

Detached-leaves of *Pisum sativum*, *Ginkgo biloba* and hydrated thalli of *Parmelina tiliacea* were kept in the dark immediately for 15 min at room temperature and then submerged in water of 25 or 50°C for 40 s. Following the heat treatment, the samples were taken out of the water and after 5 min of acclimation to the room temperature conditions, PF, DF, MR were measured. We noted here, that the DF was recorded in the dark time range of 10–30 μs . To ensure the reproducibility of the measurements, a minimum of five samples for each treatment were randomly selected. With respect to the fluorescence measurements, the fluorescence transients measured on the dry thalli did not

show any rise kinetics suggesting that the photosynthetic apparatus was in an inactive state (see [13]).

Several parameters can be derived from the PF that provide information about photosynthetic fluxes. One of the parameters calculated is the ratio $ABS/RC = [(F_K - F_o) / 0.25) \times (1/(F_J - F_o)) \times [F_M/(F_M - F_o)]$. ABS is proportional to the antenna light harvesting complex size and RC reflects the active PSII reaction center concentration. This ration indicated both the relative antenna size (proportional) and the fraction of active RCs (reciprocal) [1].

2.4. Methyl viologen (MV) treatment

MV (1,1'-dimethyl-4,4'-bipyridinium-dichloride, Fluka, Switzerland) is an efficient acceptor of electrons from the FeS-clusters of PSI and competes with ferredoxin for electrons. For the MV treatments, 200 μ M MV solution was applied to both sides of the samples with a fine brush [30] and were kept overnight in complete darkness before PF, DF, MR were measured. Each treatment was conducted with five replicates.

3. Results

3.1. Prompt and delayed fluorescence

3.1.1. Treatment at 25°C

Fig. 1 showed that following a dark-to-light transition, *Pisum sativum* and *Ginkgo biloba* leaves and thalli of *Parmelina tiliacea* exhibit a polyphasic PF rise (O-J, J-I and I-P phases) during the 1s of illumination [1] and [46]. This PF rise reveals three different reduction processes of the electron transport chain [6], [21], [47], [48] and [49]. Leaves

of *Pisum sativum* plants measured at 25°C yield a typical OJIP rise with a flat P-step (0.2–2 s). However, for *Ginkgo biloba* and *Parmelina tiliacea* thalli, the P-level was split into two peaks, the first peak P (0.2 s) is followed immediately by a decrease of the fluorescence intensity to a dip (S step) and a subsequent rise to a second peak reached after 2 s of illumination (M-peak).

The DF induction curve measured at 10–30 ms dark interval consists of a rise to a peak I_1 (at 7 ms), a subsequent decrease through I_2 (at about 100 ms), and finally I_4 peak at 0.5–5 s. I_1 peak is between the J-step towards the I-step of the PF curve, I_2 appeared in the range of the I–P phase and I_4 appeared during the decline of the P–S–M phase. We note here that the peak I_4 appeared clearly in DF curve of *Parmelina tiliacea* thalli at 0.7 s and at 1.5 s in *Ginkgo biloba* leaves and an antiparallel relation between J–I–P–S and DF curves was observed.

It is known that MV is an efficient electron acceptor, which competes with Fd for electrons at the acceptor side of PSI. Here, we examined whether the P–S–M rise and I_4 -peak from DF is sensitive to MV treatment. In samples of the three plant species treated with MV, the amplitude of the I–P phase of the PF curve was reduced and this change was accompanied by a disappearance of the PSM transient. Further, in the presence of MV, the DF intensity decreased; and the I_4 peak disappeared (Fig. 1). The induction curves both PF and DF in the tree investigated samples became similar.

3.1.2. Treatment at 50°C

Heat treatment at 50°C induced a change in the shape of PF and DF curves and their intensities (Fig. 2). A decrease in F_M and increase in F_0 and suppression of the J–I phase

of fluorescence rise kinetic were also observed in the PF transients of the three plant species. Furthermore, an additional K step at 0.3 ms was observed and a second peak equates biochemically the P step referring to Tóth et al. [50]. The appearance of K-band has been associated with a dissociation of the oxygen-evolving-complex and it consists of a fast rise with a maximum at 300 μ s followed by a reduction in fluorescence intensity as a result of photoinduced formation of fluorescence quenching RCs with oxidized P680. Indeed, the appearance of K-band is considered as a major change in OJIP rise kinetics under high temperature. This second peak was emerged at $\sim$ 1s in *Pisum sativum* leaves and *Ginkgo biloba* was also observed. In the case of *Parmelina tiliacea*, we observed some heat-induced changes that differed from those observed in *Pisum sativum* and *Ginkgo biloba* leaves. These changes are as follows: the second peak around P step was near absent; heat treatment enhanced the fluorescence rise from S to M and also a prominent peak around the I-step is observed. These results demonstrate that in all three plant species, 50°C incubation provoked a reduced electron transport rate through PSII.

With respect to DF measurements, heat treatment at 50°C provoked a decrease in its intensity and a change in its shape compared to the 25°C treatment (Fig. 2). A careful analysis of the DF curves recorded in heat-treated samples showed that the 50°C treatment shifted the I₁ step in DF curve to shorter times (from 7 ms to 2-3 ms) and shifted I₂ to longer times (from 100 ms to 200-300 ms). The position of I₄ did not change in *Ginkgo biloba* leaves. However, the I₄ peak disappeared in heat-treated *Parmelina tiliacea* thalli.

In heat-treated *Pisum sativum* leaves, PF intensity increase up to the K-peak and decreased again; in contrast, the DF intensity increased. In *Ginkgo biloba* leaves, we

observed a close anti-parallelism between changes in PF and DF. However, this anti-parallel relationship between PF and DF was lost in *Parmelina tiliacea*. In MV + 50°C treatment, the DF intensity decrease in thalli of *Parmelina tiliacea* and the I₄ peak disappeared from DF curve when compared with measurements made on thalli kept at 25°C (Fig. 2).

The increase of the absorbed energy by active RCs (ABS/RC) was observed in the three plants species exposed to 50°C and an increase in this parameter means that a fraction of RCs was inactivated and thalli of *Parmelina tiliacea* presented the high fraction of inactivated RCs compared to *Pisum sativum* and *Ginkgo biloba* heated-leaves (Fig. 3).

3.1.3. Modulated 820 reflection

We also investigated heat-induced changes in the MR-kinetics. In the three plant species, an MR decrease - reflecting a photoinduced oxidation of P700 and PC, i.e. an increase of the P700⁺ and PC⁺ population - is observed until about 20 ms of illumination (Fig. 4). This time interval equates the O-J-I phase of PF. Subsequently, an MR increase is observed, reflecting the re-reduction of both P700⁺ and PC⁺ by intersystem electrons. This process equates the I-P phase of PF [21]. For *Parmelina tiliacea* kept at 25°C the I-P-S-M transition follows the reduction and oxidation of PC and P700, whereas an increase in the MR-signal paralleling the S-M rise did not occur in *Parmelina tiliacea* thalli treated at 50°C. After 50°C incubation, the re-reduction of P700⁺ and PC⁺ started earlier and began already after 10 ms of illumination. We note here that treatment with MV led to a disappearance of the re-reduction of both P700⁺ and PC⁺ and eliminated all further kinetics (data not shown).

4. Discussion

Short-term treatment of the three plant species to 50°C induces changes on the PF, DF and 820 nm reflection (MR) signals (Figs.1, 2 and 4). The shape of the Chl *a* fluorescence recorded was changed and a strong decrease of fluorescence yield at J, I and P phases of the fluorescence transitions compared to 25°C treatment was observed. This indicates that a strong inhibition of electron transport from OEC toward PQ pool was occurred. Early studies with regard to the heat-induced damage of PSII found that heat treatment leads to various damages, such as inhibition of donation of electrons by OEC [51] and [52]; inhibition of the electron flow from Q_A to Q_B [53]. Here, the increase of F_o at 50°C compared to treatment at 25°C has been related to physical separation of the PSII from associated pigment antennae, decrease of excitation energy trapping in PSII reaction centers and emission of part of unused in photochemistry energy as fluorescence emission. The reduced flux of trapped energy and weakened electron donation from the oxidizing side caused by OEC inhibition leads to suppression of the J-I phase [43] and [54].

In addition, the decrease of F_M at 50°C compared to treatment at 25°C could be due to an inhibition of the PSI acceptor side (Fig. 4 and see [21]). Fig. 2 showed that PF, DF and MR in heated samples of *Parmelina tiliacea* thalli seemed susceptible to heat-injury compared to heated-leaves of *Pisum sativum* and *Ginkgo biloba* plants. Indeed, thalli of *Parmelina tiliacea* PF, DF and MR signals were strongly affected by heat treatment compared to *Pisum sativum* and *Ginkgo biloba* leaves.

On the one hand, PF rise from F_o to F_M was related to a gradually closed of RCs [47] and the observed antiparallel relation between J-I-P-S and DF measured at 25°C suggest that

DF can be related to the open RCs (see also [33] and [34]). With respect to DF recorded after 50°C incubation, a decrease in its intensity was noted as well as in PF and this indicates a causal relationship between DF and redox state of the electron transport. It is recognised that heat treatment induced an inhibition of the charge-separation in the PSII reaction center [55]. Then, the decrease in active RCs at 50°C provoked a decrease of the DF intensity in the three plant species. Indeed, in a previous, Čajánek et al. [56] showed that DF signal measured in heated barley leaf segments at 42.5-45°C decreased by more than 70% compared to DF measured at 40°C. These authors noted that this decrease was related to irreversible PSII damage which abolished the recombination of separated charges ($P680^+ Q_A^-$). Indeed, the first reduction of Q_A and the subsequent reduction of $P680^+$ require one electron which is normally present on Y_Z [57]; and there is no necessary involvement of the OEC.

After light-induced charge separation between P680 and Pheo, the first reduction of Q_A occurred and P680 was reduced by one electron present on Y_Z [57]. At 50°C treatment, the fluorescence rise at K-step is the result of only one stable charge separation that lets to Q_A^- accumulation and the dip after the K-peak was caused by oxidation of Q_A^- , which can not be re-reduced due to the destruction of OEC [43]. This deficient electron donor side would lead to the accumulation of $P680^+$. However, the shift of I_1 step at 50°C in DF curve to shorter time from 7 ms at 25°C to 2-3 ms (see Figs. 1 and 2) might be due a rapid recombination between Q_A^- and $P680^+$ and to inefficient light-induced charge separation between P680 and Pheo (damage of OEC). On the other hand, the shift I_2 in DF curve to longer time (from 100 ms to 200-300 ms) is due to prolonged closed PSII RCs due to inhibition of electron transfer from Q_B to PQ. Further, the fact that MV

treatment disappeared I₄-peak in DF emission; permit us to suggest that the I₄-peak observed at 25°C in thalli of *Parmelina tiliacea* and *Ginkgo biloba* leaves was caused by a temporary increase of a cyclic electron transport flow from the PQ-pool to PSI.

It has been reported that the slow shape of P-S-M-T phases from Chl *a* fluorescence varies in different organisms and depends on their nature and history [58]. This slow P-S-M-T phase is complex, because Calvin–Benson cycle and several other correlated physiological processes take place within this timescale [59]. Several approaches and explanations of P-S-M-T transients were proposed. The P step in PF that splits into two peaks with maxima at about 0.2 and 2 s has been reported in *Pinus brutia* needles to link to the activation kinetics of the enzyme FNR that differs between species [11]. Previous studies showed that in angiosperm species such as pea plants the activation of the acceptor side of PSI, i.e. the activation of FNR, is much slower than in gymnosperm species or lichens. The fast activation of FNR makes an additional process visible reflected by the M-peak [11], [12] and [13]. Furthermore, it has been reported that the S-M phase increased when the state transitions become dominant [14], [59], [60] and [61]. Also Papageorgiou et al. [7] argued that differences in thylakoid membrane structure and stacking may reflect in P-S-M pattern. In the present study, the decrease of fluorescence from P peak to S in unstressed (25°C) of *Ginkgo biloba* and *Parmelina tiliacea* coincides with an increase in DF intensity during part of I₄ peak (Fig. 1) and by an oxidation of P700 and PC (Fig. 4). Then, P to S rise involved a re-oxidation of Q_A by PSI. In addition, MV treatment abolished P-S-M rise (in both stressed and unstressed samples) and therefore, we suggested that P-S-M rise from PF might be related an acceleration of intersystem electron transport.

It is known that the redox state of PQ pool located between the two photosystems determines the activity of LHC-II kinase and plays a regulatory mechanism in excitation energy distribution between the two photosystem in favour to PSII [62]. Here the full reduction of PQ pool did not occurred after 50°C incubation caused by weaker electron flow from PSII (disappearance of the J–I phase that has been reported to be related to the reduction of the PQ). However, the peak around the I-step shown in Fig. 2 in *Parmelina tiliacea* indicates that PQ pool would be more reduced in darkness following a 50°C heat treatment (see [13] for more details).

Based on the work of Schansker et al. [30] the PQ-pool is largely reduced around the I step in OJIP transient. Under those conditions an increase in the DF intensity can only happen if the population of electron holes (i.e. $P680^+$) on the donor side of PSII increases. This is only likely to happen if the population of PSII RCs in the S_3 state increases again. But due to the desynchronisation of the S-states occurring during the fluorescence rise as a function of the number of charge separations, this peak is expected to be strongly dampened and therefore to be associated with a much lower DF intensity as is observed in Fig. 1. Furthermore, following the destruction of the Mn-cluster by high temperature only Tyr_Z can donate an electron to $P680^+$ [54] and it was observed that the lifetime of Tyr_Z⁺ becomes much longer and the concentration of $P680^+$ will be relatively high. This means that the intensity of the DF-signal will be determined mainly by the Q_A^- concentration. Oukarroum et al. [13] proposed that *Parmelina tiliacea* lacked alternative electrons donors to PSII (e.g. re-reduction of Tyr_Z by ascorbate) when the OEC had been destroyed by heat stress. The lack of alternative electrons donors to in *Parmelina tiliacea* at 50°C was showed by an absence of P-peak in PF compared to DF in pea and *Ginkgo*

biloba heated-leaves (see Fig. 2 and [13]). However, internal alternative electron donors to PSII might play a role in DF induction.

The changes in DF intensity during the induction period are related with the build-up of the electrical and proton transmembrane gradient [63]. Also it has been reported that under heat treatment, it was suggested that to maintain a proton gradient, ATP synthesis was induced by cyclic electron flow around PSI to balance ATP consumption when linear electron transport is inhibited [64], [65] and [66]. The reduction in proton gradient might be decreased DF observed in Fig. 2. To support this idea, a decrease in delayed luminescence was observed in *Chlorella pyrenoidosa* after addition of tri-*N*-butyl-tin (TNBT) -an inhibitor of ATPase activity [67]. The inhibition of ATPase activity by TNBT has consequently a prevention of the proton gradient and membrane potential and then reduction in the rate of charge recombination between the oxidized donor and reduced acceptor of PSII centers.

In this study, the decrease in DF intensity recorded after 50°C incubation in the three plant species as well as in PF intensity indicates a causal relationship between DF and redox state of the electron transport. Indeed, inhibition of the charge-separation in the PSII reaction center induced by heat treatment decreased active RCs (the OEC is destroyed) and then a decrease of the DF intensity.

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Figure legends

Figure 1. Simultaneous measurements of prompt fluorescence (closed symbol) and delayed fluorescence (open symbol). Measurements induced by a 10 s pulse of strong red actinic light (627 nm peak, 5000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in *Pisum sativum* and *Ginkgo*

biloba leaves and thalli of *Parmelina tiliacea*. Samples were heated at 25°C for 40 s and treated or not by methyl viologen (MV) (see Materials and methods). Transients are averages of five independent measurements.

Figure 2. Simultaneous measurements of prompt fluorescence (closed symbol) and delayed fluorescence (open symbol). Measurements induced by a 10 s pulse of strong red actinic light (627 nm peak, 5000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in *Pisum sativum* and *Ginkgo biloba* leaves and thalli of *Parmelina tiliacea*. Samples were heated at 50°C for 40 s and treated or not by methyl viologen (MV) (see Materials and methods). Transients are averages of five independent measurements.

Figure 3. Relative ratio of the light harvesting complex absorption per PSII active reaction centers (ABS/RC) of heat-treated sample of *Pisum sativum*, *Ginkgo biloba* and thalli of *Parmelina tiliacea*.

Figure 4. Kinetics of modulated reflection at 820 nm (MR) induced by red actinic light of 5000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in *Pisum sativum*, *Ginkgo biloba* and thalli of *Parmelina tiliacea* samples kept in dark for 15 min and heated to 25 or 50°C for 40 s.

Figure 1

Figure 2

Figure 3

Figure 4