

# Probing the FQR and NDH activities involved in cyclic electron transport around Photosystem I by the ‘afterglow’ luminescence

Michel Havaux<sup>a,\*</sup>, Dominique Rumeau<sup>a</sup>, Jean-Marc Ducruet<sup>b</sup>

<sup>a</sup>CEA/Cadarache, DSV, DEVM, Laboratoire d'Ecophysiologie de la Photosynthèse, UMR 6191 CNRS-CEA-Aix Marseille II, F-13108 Saint-Paul-lez-Durance, France

<sup>b</sup>Service de Bioénergétique, INRA/CEA-Saclay, F-91191 Gif-sur-Yvette cedex, France

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## Abstract

Far-red illumination of plant leaves for a few seconds induces a delayed luminescence rise, or afterglow, that can be measured with the thermoluminescence technique as a sharp band peaking at around 40–45 °C. The afterglow band is attributable to a heat-induced electron flow from the stroma to the plastoquinone pool and the PSII centers. Using various *Arabidopsis* and tobacco mutants, we show here that the electron fluxes reflected by the afterglow luminescence follow the pathways of cyclic electron transport around PSI. In tobacco, the afterglow signal relied mainly on the ferredoxin-quinone oxidoreductase (FQR) activity while the predominant pathway responsible for the afterglow in *Arabidopsis* involved the NAD(P)H dehydrogenase (NDH) complex. The peak temperature  $T_m$  of the afterglow band varied markedly with the light conditions prevailing before the TL measurements, from around 30 °C to 45 °C in *Arabidopsis*. These photoinduced changes in  $T_m$  followed the same kinetics and responded to the same light stimuli as the state 1–state 2 transitions. PSII-exciting light (leading to state 2) induced a downward shift while preillumination with far-red light (inducing state 1) caused an upward shift. However, the light-induced downshift was strongly inhibited in NDH-deficient *Arabidopsis* mutants and the upward shift was cancelled in plants durably acclimated to high light, which can perform normal state transitions. Taken together, our results suggest that the peak temperature of the afterglow band is indicative of regulatory processes affecting electron donation to the PQ pool which could involve phosphorylation of NDH. The afterglow thermoluminescence band provides a new and simple tool to investigate the cyclic electron transfer pathways and to study their regulation in vivo.

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**Keywords:** *Arabidopsis*; Cyclic electron transport; Ferredoxin-plastoquinone oxidoreductase; NAD(P)H dehydrogenase; Photosystem I; Thermoluminescence

## 1. Introduction

Far-red light induces a long-lived luminescence in plants, which manifests as a bounce superimposed on the luminescence decay in the dark [1,2]. This afterglow (AG) emission, although induced by far-red light which prefer-

entially excites PSI, originates from PSII [3]. It is attributed to a back-flow of electrons from reducing compounds in the stroma to the plastoquinones (PQ) and the quinonic electron acceptors of PSII, oxidized by far-red light [4,5]. The reduced  $Q_B^-$  acceptors thus formed in the dark can recombine with the S2 and S3 states of the manganese oxygen-evolving complex of PSII, leading to light emission. In agreement with this interpretation, temperature elevation up to 40–45 °C has been shown to stimulate electron donation from the stroma to the acceptor side of PSII [6–8] and to enhance the AG emission [1,9]. Because the AG is stimulated by increasing temperature, this signal can be conveniently measured by thermoluminescence (TL), as a sharp band peaking between 40 and 50 °C

**Abbreviations:** AG, afterglow; B-band, thermoluminescence band at ca. 30 °C due to S2/3  $Q_B^-$  recombination; PQ, plastoquinone; PS, photosystem;  $T_m$ , temperature of the maximum of the AG band; TL, thermoluminescence; E, Emerson enhancement; FQR, ferredoxin-quinone oxidoreductase; NDH, NAD(P)H dehydrogenase; PDF, photon flux density

\* Corresponding author. Tel.: +33 4 4225 7476; fax: +33 4 4225 6265.

E-mail address: [michel.havaux@cea.fr](mailto:michel.havaux@cea.fr) (M. Havaux).

[10,11]. The AG TL band corresponds to a heat-induced stimulation of electron transfer from stromal reductants to PSII centers that are initially in the non-recombining state  $S_2/3 Q_B$  and are progressively converted to light-emitting  $S_2/3 Q_B^-$  states.

An important feature of the AG is its inhibition by antimycin [12] and its acceleration by N-methylphenazonium metosulphate [2], which are, respectively, an inhibitor and an activator of cyclic electron transport around PSI in chloroplasts. This suggests that the electron fluxes responsible for the AG and the cyclic electron transfer chain share a common sequence of electron carriers. In agreement with this idea, induction of cyclic electron transport by environmental stress conditions, as probed by  $P700^+$  reduction and photoacoustic measurements, was found to be correlated with an acceleration of the AG luminescence in maize [13]. In green plants, electron recycling from the stroma-exposed side of PSI to the PQ pool can occur via two different routes [14–16]. One route is mediated by NAD(P)H and involves the chloroplastic NAD(P)H dehydrogenase (NDH) complex [17–19] which participates also in chlororespiration [20]. The other cyclic pathway is sensitive to antimycin and involves a putative ferredoxin-plastoquinone oxidoreductase (FQR) that catalyzes electron transfer from ferredoxin to the PQ pool [19,21]. In this study, the suggested relationship between AG luminescence and cyclic electron flow was analyzed using thermoluminometry in a series of tobacco and *Arabidopsis* mutants affected in the cyclic electron transfer pathways. The presented results show that the AG requires the activation of an electron cycle around PSI. The heat-induced electron flux reflected by the AG TL band is mediated by both the FQR and the NDH enzymatic complexes. The relative contribution of each pathway varies, however, from one species to the other.

## 2. Materials and methods

### 2.1. Plant material

Wild-type tobacco (*Nicotiana tabacum*, cv. Petit Havana) and the *ndhB*-inactivated mutant [22] were grown in a phytotron, as previously described [18]. Wild-type *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh., cv. Colombia, Landsberg erecta or Wassilewskija) and the *ndh* and *ndh* mutants were grown under controlled conditions, as previously described [23]. A complete description of both *ndh* mutants can be found in Rumeau et al. [24]. The *pgr5* mutant, the *crr4-2* mutant and the double mutant *pgr5 crr4-2* were grown under similar conditions except that the photon flux density was reduced to  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  because the *pgr5* mutant is photosensitive. Both mutations are described in Munekage et al. [16,19]. The cyanobacterium *Synechocystis* PCC6803 was

grown at 30 °C in Allen's medium as described elsewhere [25].

### 2.2. Treatments

The lower epidermis of tobacco leaves was stripped off as described in [17]. Stripped leaf discs were soaked in Petri dishes containing water and 5  $\mu\text{M}$  antimycin (Sigma). Time of incubation was ca. 30 min. We did not succeed in peeling *Arabidopsis* leaves. Therefore, *Arabidopsis* leaf discs were soaked in water with 5  $\mu\text{M}$  antimycin for minimum 3 h under gentle stirring. With intact tobacco leaf discs, this 3-h treatment gave similar results as 30-min floating of stripped leaf discs on 5  $\mu\text{M}$  antimycin. The AG band was abolished in leaf discs infiltrated under vacuum with water or with a buffer. This confirms that the AG emission occurs in intact systems only (isolated thylakoid membranes do not emit an AG luminescence). Therefore, it was not possible to use the vacuum technique to infiltrate leaves with antimycin. Detached *Arabidopsis* leaves placed on wet filter paper were driven to the light state 2 by illumination at 25 °C with a blue-green light produced by metal halide lamps (Osram, Powerstar HQI-TS, 150 W/NDL) and a BG18 Schott filter (PFD, 100 or 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Far-red light (30  $\text{W m}^{-2}$ ) obtained by filtering the light through a RG715 Schott filter was used to drive leaves to state 1.

### 2.3. Thermoluminometry

The luminescence emitted by leaf discs (diameter, 12 mm) or cyanobacterial cells deposited on a nitrocellulose filter (diameter, 1 cm) was measured with a custom-made apparatus that has been described in detail elsewhere [26]. In brief, the sample was placed in the bottom of a cylindrical cuvette (diameter, 25 mm) made from heat-resistant plastic against a thin aluminium plate (1 mm), the temperature of which was regulated with a water-cooled Peltier system (40 × 40 mm Thermatec Peltier plate from Melcor) powered by a variable 0 to 5A current. Temperature was measured with a tiny thermocouple inserted in the aluminium plate at the center of the Peltier element at 0.5 mm under the sample. A drop of water (100  $\mu\text{l}$ ) was placed on the center of the aluminium plate to ensure good thermal contact with the leaf. The sample was maintained at 0 °C or 10 °C for 30 s in the dark, then it was illuminated for 60 s with far-red light (>715 nm) at this temperature. Far-red light, produced by a Schott KL1500LCD light source and a RG-715 Schott filter, was passed onto the sample with one arm of a 4-arm light guide (Walz). The fluence rate of the far-red light, measured with a Li-Cor Li185B/Li200SB radiometer, was about 50  $\text{W m}^{-2}$ . Immediately after switching off the far-red light, temperature was increased from 0 °C or 10 °C to 70 °C, at a rate of 0.5 °C  $\text{s}^{-1}$ . The

luminescence emission by the sample was measured during heating by a photomultiplier tube, protected by a red filter, via one arm of the guide. The photomultiplier was a compact Hamamatsu H5701-50 photomultiplier module with built-in amplifier and with a  $\pm 15$  V power supply. The instrument was driven and the temperature/light signals were recorded by a PC computer equipped with a National Instruments DaqPad-1200 connected to the parallel port of the computer.

#### 2.4. Photoacoustics

The changes in light distribution between PSI and PSII during the state transitions were estimated by the Emerson enhancement (E) of photosynthetic oxygen evolution, using the photoacoustic technique [27]. The photoacoustic spectrometer and the procedure used to separate the photobaric photoacoustic signal (due to modulated oxygen evolution) from the photothermal photoacoustic signal have been described in detail elsewhere [23]. Modulated oxygen evolution was induced by a blue-green light ( $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) modulated at 19 Hz. Light state 2 was reached after 20-min illumination with this modulated light. Because PSII absorbs more blue-green light than PSI, oxygen evolution is limited by the light absorption by PSI. The increase in modulated oxygen evolution upon adding a strong, nonmodulated far-red light absorbed almost exclusively by PSI is called the ‘Emerson enhancement’ and corresponds to the removal of this limitation. Thus, the extent of the far-red light-induced enhancement of oxygen evolution is a measure of the imbalance in light energy distribution in favor of PSII. During the transition to state 2, LHCII migrates from PSII to PSI, hence increasing the absorption cross-section of PSI (and decreasing that of PSII) and resulting in a low E value. Transition from

state 2 to state 1 is associated with the migration of LHCII back to PSII, leading to an unbalanced distribution of the blue-green light between PSI and PSII in favor of the latter one. Therefore, state 1 is characterized by high E values.

### 3. Results

#### 3.1. The AG TL band in tobacco and *Arabidopsis* leaves

Tobacco plants were kept for about 30 min in dim light ( $5\text{--}10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) before the experiments. When leaves taken from such plants were illuminated with far-red light at  $0^\circ\text{C}$  and subsequently warmed in the dark, a burst of photons was observed in the temperature range  $30\text{--}50^\circ\text{C}$  with a maximum photon emission at ca.  $42^\circ\text{C}$  (Fig. 1A). This sharp TL band corresponds to the AG luminescence. A previous work has shown that it exhibits all the basic properties of the afterglow emission measured as a transient rise in the post-far-red luminescence decay [11]. No such band was observed without prior far-red light excitation (Fig. 1A) or in leaves that were exposed to continuous white light at  $0^\circ\text{C}$  (data not shown). The AG band measured in tobacco leaves at around  $42^\circ\text{C}$  after far-red illumination was usually preceded by a smaller peak at ca.  $19^\circ\text{C}$  which has been identified as a B band due to recombination of preexisting  $S_2/3 Q_B^-$  PSII centers [11]. This B-type band was downshifted from its standard temperature ( $35^\circ\text{C}$ , see [28]) to  $19^\circ\text{C}$ , presumably because of the acidic pH of the lumen induced by far-red light [11]. The far-red light-induced AG TL band of *Arabidopsis* leaves was very similar to the AG signal obtained with tobacco leaves, with the AG band peaking at  $42^\circ\text{C}$  and the downshifted B band appearing at  $16^\circ\text{C}$  (Fig. 1B).

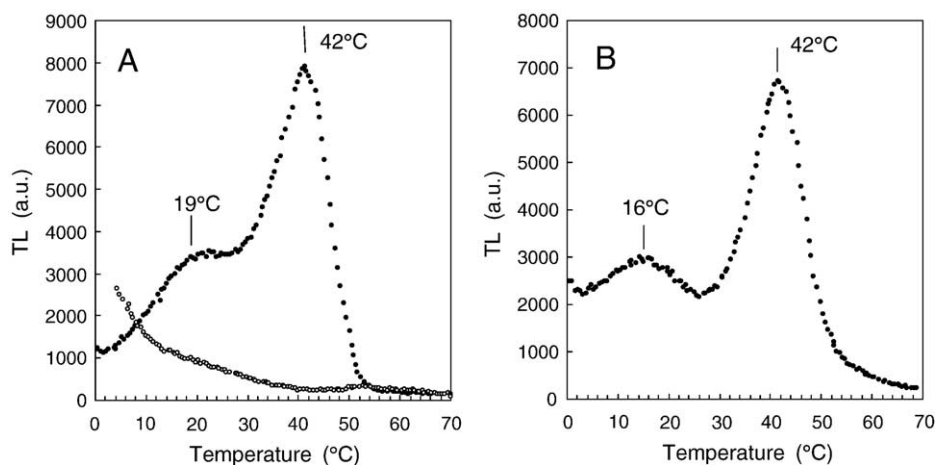


Fig. 1. (A) TL signal of a tobacco leaf disc illuminated for 0 or 60 s (open or closed circles, respectively) with far-red light at  $0^\circ\text{C}$ . The  $42^\circ\text{C}$  peak is the so-called afterglow luminescence (AG). (B) TL signal of an *Arabidopsis* leaf disc (WT, ecotype Columbia) after 60 s far-red light at  $0^\circ\text{C}$ .

### 3.2. AG luminescence in tobacco mutant leaves

Inactivation of the chloroplastic NDH complex in the *ndhB* tobacco mutant did not affect significantly the amplitude of the AG band (Fig. 2A). In contrast, the AG TL band amplitude was drastically reduced in leaves treated with 5  $\mu$ M antimycin, a well-known inhibitor of the FQR pathway of cyclic electron transport around PSI [21]. Only a very small peak at 42 °C remained after the antimycin treatment (Fig. 2B), suggesting that most of the electrons that led to the heat-induced AG emission in tobacco leaves were transferred to  $Q_B$  from the stroma via the FQR. Interestingly, the effect of the antimycin treatment was even more pronounced in the *ndhB* mutant, resulting in a complete suppression of the luminescence peak at 42 °C (Fig. 2C). This suggests that another pathway, presumably the NDH-dependent pathway, is also involved in the AG signal. However, it is clear that, in tobacco leaves, the participation of this pathway is very small compared to the FQR pathway. The complete suppression of the AG in the *ndhB* mutant treated with antimycin (Fig. 2C) demonstrates that the AG luminescence emission is strictly dependent on the cyclic electron

transport pathways. It should be noticed that the B band was less downshifted (22 °C instead of 16 °C in control WT) by the far-red illumination in antimycin-treated leaves, presumably due to a slower proton influx in the lumen under far-red light when the FQR pathway is blocked.

### 3.3. AG luminescence in *Arabidopsis* mutant leaves

Contrary to tobacco, AG luminescence in *Arabidopsis* leaves showed little sensitivity to antimycin; the AG band amplitude was only slightly reduced by 5  $\mu$ M antimycin (Fig. 3A). The strong inhibitory effect of antimycin on the *crr4-2* mutant (see below) confirmed that antimycin did penetrate into the chloroplasts. Also, the *pgr5* *Arabidopsis* mutant, which is affected in a component of the antimycin-sensitive route of cyclic electron transport [19], exhibited an AG signal quite similar to that of WT leaves (Fig. 3B). In contrast, the AG emission from leaves of the *crr4-2* *Arabidopsis* mutant, which is characterized by a drastic reduction of the NDH activity to 12–15% of the WT level [16], had a strongly reduced amplitude (Fig. 3B). This effect was confirmed in two other *ndh* mutants, a *ndhO* mutant and

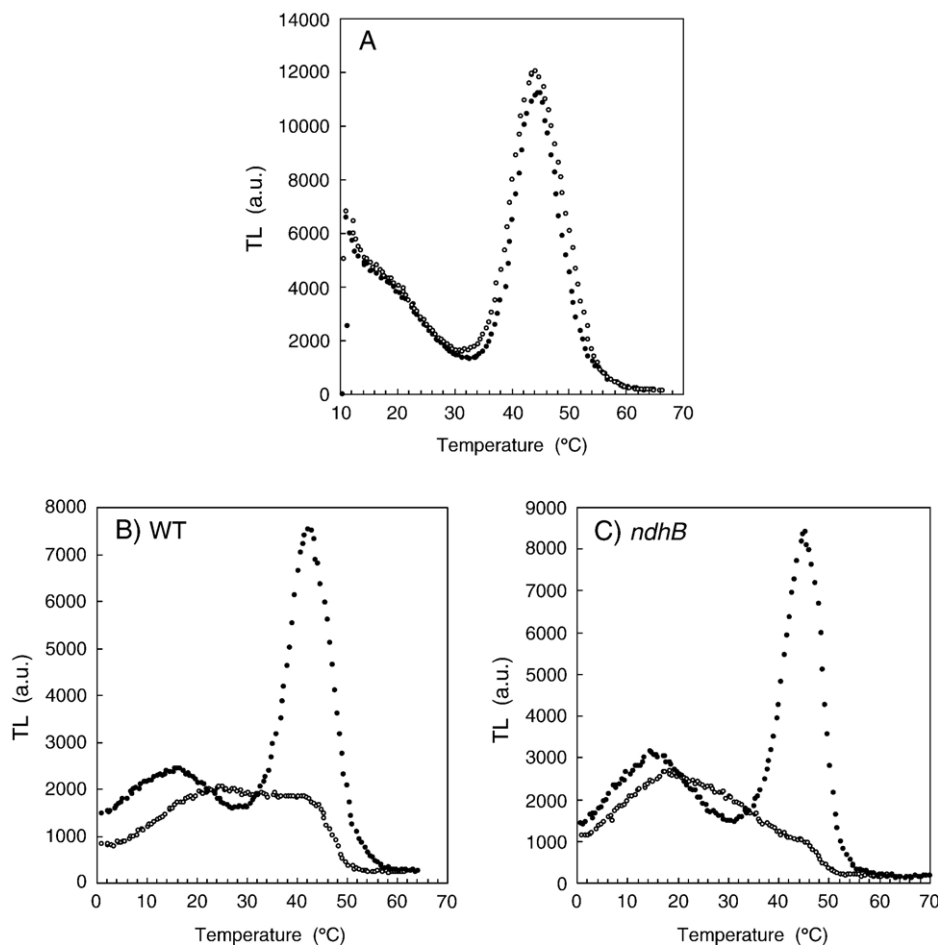


Fig. 2. (A) Far-red light-induced AG TL band of WT (closed circles) and *ndhB* mutant (open circles) tobacco leaves. (B–C) Far-red light-induced AG TL band of WT and *ndhB* mutant tobacco leaves infiltrated with water (closed circles) or 5  $\mu$ M antimycin (open circles).

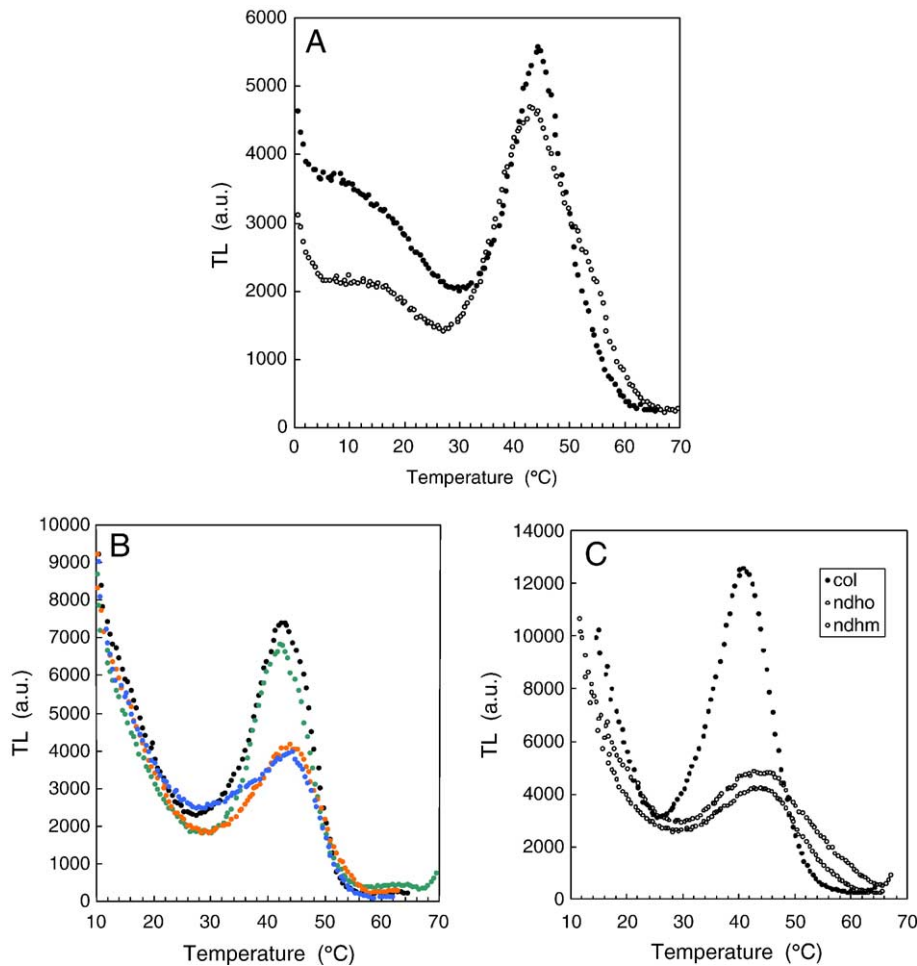


Fig. 3. (A) Far-red light-induced AG TL band of WT *Arabidopsis* leaves infiltrated for 3 h with water (closed circles) or 5  $\mu$ M antimycin (open circles). (B) Far-red light-induced AG TL band of WT *Arabidopsis* (Columbia, in black) and a series of mutants: *pgr5* (green), *crr4-2* (red) and *crr4-2 pgr5* (blue). (C) Far-red light-induced AG TL band of WT *Arabidopsis* (Columbia) and the *ndho* and *ndhn* mutants. The AG TL band of ecotype *erecta* (not shown) was similar to the AG signal of Columbia.

a *ndhn* mutant, which are both completely devoided of the chloroplastic NDH complex [24] (Fig. 3C). When the *pgr5* mutation was combined with the *crr4-2* mutation, no effect of the former mutation could be seen: the AG emission from the *crr4-2* single mutant and the *pgr5 crr4-2* double mutant were virtually identical (Fig. 3B).

The small AG signal present in the *crr4-2* mutant was sensitive to antimycin (Fig. 4). Suppression of the AG band by antimycin was also found in the *ndho* and *ndhn* mutants (data not shown). Thus, we can conclude that the FQR is also involved in the *Arabidopsis* AG signal, but its contribution seems to be minor compared to the NDH complex. The same inhibitory effect of antimycin was observed in the double mutant *pgr5 crr4-2* (Fig. 4). Thus, the effect of the *pgr5* mutation and the antimycin treatment on the AG is not comparable, although the product of the *pgr5* gene is supposed to be a component of the antimycin-sensitive cyclic pathway [19].

The measurements done on *Arabidopsis* corroborate the results obtained with tobacco since, in both species, the AG emission required an active electron cycle around PSI.

However, the relative involvement of the NDH and FQR complexes in the flux leading to the AG luminescence appeared to be very different in *Arabidopsis* and tobacco.

#### 3.4. Light-induced downshift of the AG band

The peak temperature ( $T_m$ ) of the AG TL band was observed to vary with the leaf history. Prolonged dark adaptation of tobacco or *Arabidopsis* leaves (e.g., one night) rose the peak temperature from 42 °C to 45 °C (data not shown). In a previous study of maize leaves, we observed that the AG peak temperature shifted towards lower temperatures by several degrees in response to light pretreatments that supposedly favored cyclic electron transfer around PSI [13]. In *Arabidopsis* (Fig. 5A) and, to a lesser extent, in tobacco (data not shown), adaptation to white light prior to the TL measurements induced a downshift of the AG band.

Fig. 5B shows that very large variations of  $T_m$  can be induced by illuminating the leaves with PSII-exciting or PSI-exciting light beams at 25 °C. Far-red light, which

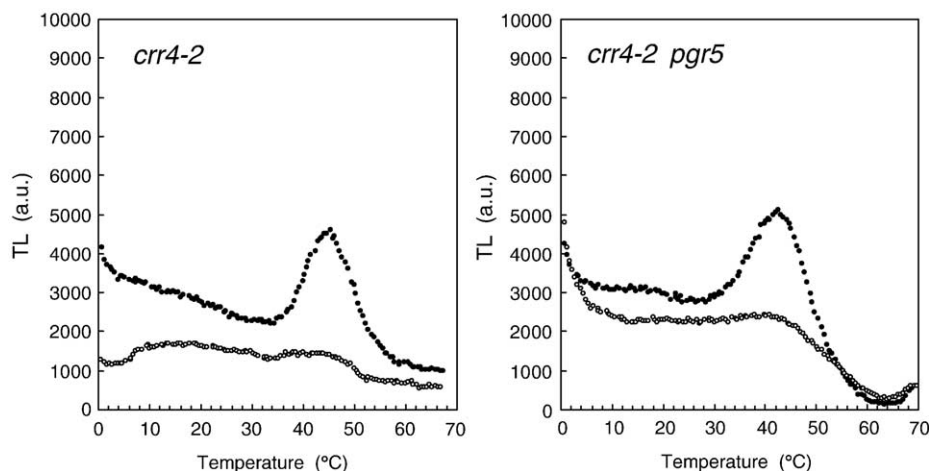


Fig. 4. AG TL band of *crr4-2* and *crr4-2 pgr5* mutant *Arabidopsis* leaves infiltrated with water (closed symbols) or with 5  $\mu\text{M}$  antimycin (open symbols).

excites almost exclusively PSII and drives leaves to the so-called state 1, caused  $T_m$  to increase up to 45  $^{\circ}\text{C}$  within a few minutes. Conversely, blue-green light exciting preferentially PSII and driving the leaf to state 2 brought about a marked reduction of  $T_m$  from 45  $^{\circ}\text{C}$  to 30  $^{\circ}\text{C}$  within approx. 10 min. Fig. 5C shows that the changes in light energy distribution between the two photosystems, as measured by the Emerson enhancement, during a far red-light-induced

transition from state 2 to state 1 proceeded with a kinetics similar to that of the photoinduced AG shift. Thus, the light-state transitions and the photoinduced shift of the AG band seem to respond to the same stimuli with the same kinetics. This suggests that the position of the AG band is determined by the state transition phenomenon or by another phenomenon governed by the reduction/oxidation level of the PQ pool.

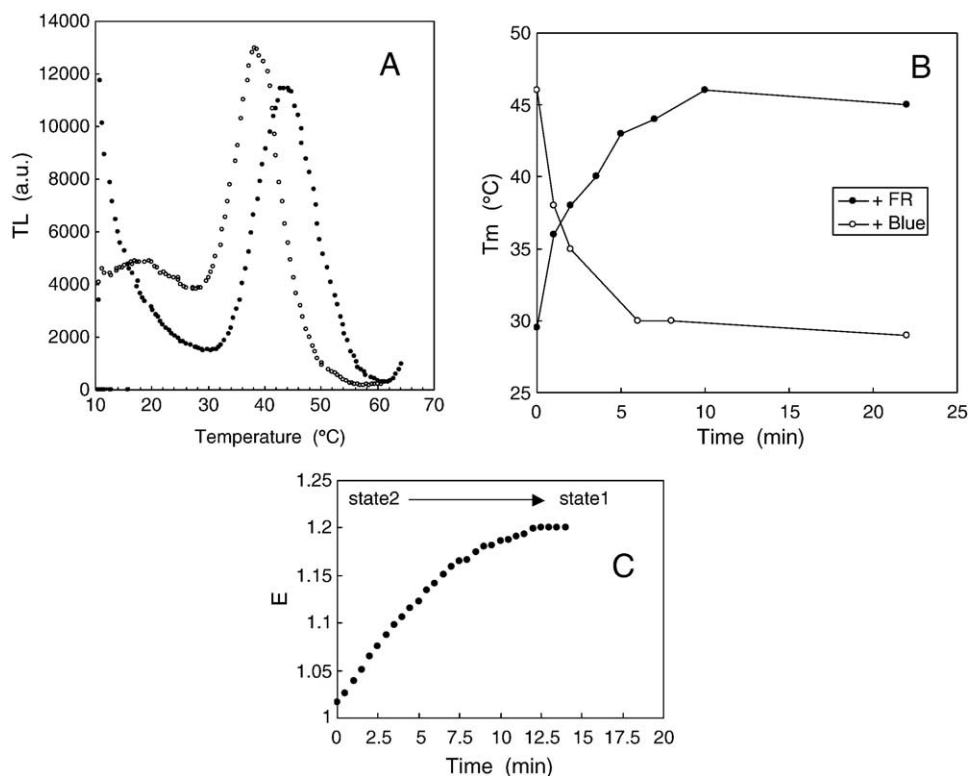


Fig. 5. (A) Far-red light-induced AG TL band in WT *Arabidopsis* leaves dark-adapted (closed symbols) or adapted to white light (open symbols, 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 30 min). (B) Peak temperature ( $T_m$ ) of the AG TL band in WT *Arabidopsis* leaves (ecotype WS) exposed to blue-green light (+Blue, open circles) or exposed to far-red light (+FR, closed circles) after 15 min in blue-green light. (C) Changes in the light energy distribution between PSII and PSI, as measured by the Emerson enhancement (E), during the transition from state 2 to state 1. The *Arabidopsis* leaf, pre-illuminated with a modulated blue-green light for 20 min (state 2), was exposed to far-red light in order to induce the conversion to state 1.

The AG band was also measured in *Arabidopsis* plants exposed for 1 week to a high PFD of  $1100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . As previously demonstrated [29,30], *Arabidopsis* is able to acclimate to those high light stress conditions, with PSII photochemistry recovering almost completely after 7–10 days. In this study, PSII photoinhibition was found to be very limited after 1 week in high light (with  $F_v/F_m \sim 0.75\text{--}0.78$ ). Interestingly, the AG band of high light-acclimated leaves was blocked at ca.  $32^\circ\text{C}$ . In contrast with control leaves, the AG band could not be shifted towards high temperatures by dark adaptation or by far-red light (Fig. 6). The photoacoustic technique was used to measure state transitions in those leaves (Table 1). The distribution of light energy between the two photosystems was quantified by the Emerson enhancement in state 2 and in state 1. 20-min illumination with blue-green light at  $25^\circ\text{C}$  (state 2) resulted in a very low  $E$  value ( $\sim 2\text{--}3\%$ ) in both control and high-light acclimated leaves, indicating, as expected, a very even distribution of blue-green light between PSI and PSII. Leaves were then driven to state 1 by adding a strong far-red light, resulting in a noticeable increase in  $E$  in control leaves and in high-light-acclimated leaves. A high  $E$  value means an unbalanced distribution of light energy in favor of PSII, as it is expected in state 1 when all LHCII are associated with PSII. Thus, both types of leaves were able to undergo state transitions and to adjust the light distribution between PSI and PSII as a function of the light quality. The maximal  $E$  value reached by the high-light-treated plants (ca. 16%) was lower than the  $E$  value measured in WT leaves (ca. 25%). This effect could be due to the selective decrease in LHCII abundance which typically accompanies growth in high light.

We have also measured  $T_m$  in *ndh* *Arabidopsis* mutants after blue or far-red illumination. The downward shift of the

Table 1

Changes in the light energy distribution between the two photosystems in *Arabidopsis* leaves (WT and *ndhO* mutant) during state transitions, as measured by Emerson enhancement  $E$

	$E$ (%)	
	In state 2	In state 1
Control WT leaves	$2.9 \pm 1.3$	$25.6 \pm 0.6$
High light-acclimated WT leaves	$2.5 \pm 1.1$	$16.8 \pm 3.8$
<i>NdhO</i> mutant leaves	$3.6 \pm 1.4$	$24.7 \pm 1.5$

Control *Arabidopsis* leaves are also compared with leaves taken from plants acclimated for 7 days to high light stress conditions ( $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $8^\circ\text{C}$  air temperature). State 2=20 min in modulated blue-green light; state 1=20 min in far-red light (see Materials and methods).

AG induced by the blue-green light was strongly inhibited in leaves of those mutants (with a maximal shift of about  $3^\circ\text{C}$ ), indicating that the NDH complex is involved in this phenomenon (Fig. 7). Incidentally, state 1–state 2 transitions were not affected by the *ndh* mutations (Table 1).  $T_m$  was also measured in tobacco. Blue-green illumination of tobacco leaves induced a decrease in  $T_m$ , but the amplitude of this decrease was smaller than that measured under the same light conditions in *Arabidopsis*, about  $4\text{--}5^\circ\text{C}$  from  $43^\circ\text{C}$  (after far-red light) to ca.  $38\text{--}39^\circ\text{C}$ . The  $T_m$  changes in tobacco leaves were inhibited by the suppression of the NDH activity (in the *ndhB* mutant) but not by antimycin (data not shown).

### 3.5. AG luminescence in cyanobacteria and green algae

No TL band in the temperature range  $30\text{--}45^\circ\text{C}$  was found in the cyanobacterium *Synechocystis* PCC6803 (data not shown). Instead, a band peaking at  $19^\circ\text{C}$  was observed, which is likely a B-type band. Similarly, no far-red light-induced AG signal was found in the green alga *Chlamydomonas reinhardtii* (not shown).

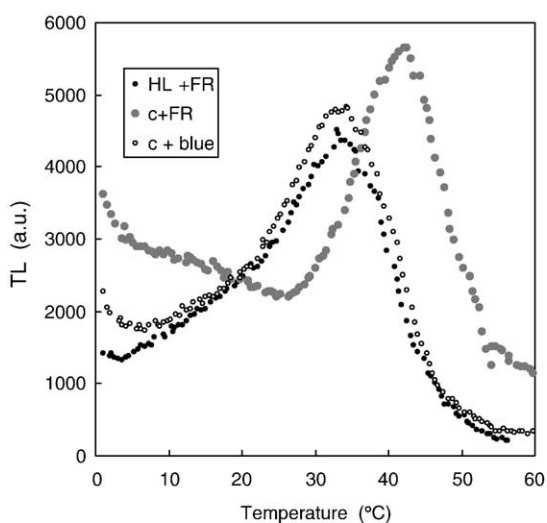


Fig. 6. AG emission from high-light acclimated *Arabidopsis* leaves preexposed to far-red light (black circles, HL+FR). For purposes of comparison, the AG signals from control leaves preilluminated with blue-green light (open circles, c+blue) or far-red light (grey circles, c+FR) are also shown.

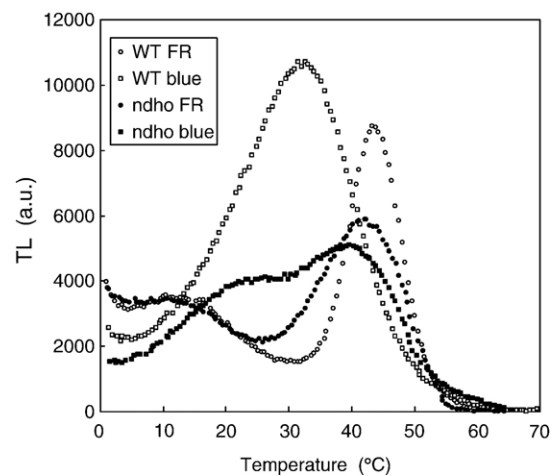


Fig. 7. AG emission of WT *Arabidopsis* and the *ndhO* mutant pre-exposed to far-red light (FR) or to blue-green light (blue).

#### 4. Discussion

This study has demonstrated that the AG luminescence induced in leaves by far-red light is related to cyclic electron transport around PSI. Block of the cyclic electron transfer fluxes was associated with a complete suppression of the AG TL band. We have shown also that the FQR complex and the NDH complex, which both mediate the electron recycling from the donor side of PSI to the PQ pool during cyclic electron transport, are involved in the electron transfer leading to the AG signal. However, the relative contribution of each complex to the AG appeared to vary substantially between species. In tobacco, FQR seems to be the predominant pathway involved in the AG emission while it plays only a marginal role in *Arabidopsis* leaves. This differential behavior of tobacco and *Arabidopsis* is consistent with previous in vitro fluorimetric measurements of PQ reducing activities in isolated thylakoid membranes. In tobacco chloroplasts, 5  $\mu\text{M}$  antimycin was observed to bring about a drastic inhibition of PQ reduction by NADPH and reduced ferredoxin to approximately 20% of the control level [31], whereas antimycin had only a very weak effect on the same activity measured in *Arabidopsis* thylakoids [16]. Conversely, deletion of the *ndhB* gene in tobacco did not affect significantly the PQ reduction rate and extent in vitro [31] while thylakoid membranes prepared from the NDH-deficient *ccr2-2 Arabidopsis* mutant exhibited a 50% inhibition of PQ reduction [16]. A similar difference between tobacco and *Arabidopsis* was observed in this study for the dependence of the AG signal on the NDH activity. Mutations that resulted in a strong or complete inhibition of the chloroplastic NDH activity had a marked effect on the AG intensity in *Arabidopsis*, but not in tobacco. Thus, the differential participation of FQR and NDH to the AG between tobacco and *Arabidopsis* likely represents a genuine difference between the activities of those enzymic complexes in the two species.

The PGR5 protein has been suggested to be a component of the FQR pathway [19]. Since the AG relies mainly on the NDH activity in *Arabidopsis*, it is not surprising that the AG signal was not perturbed in the *pgr5 Arabidopsis* mutant. However, the low-intensity AG emission that remained in the NDH-deficient *ccr4-2 Arabidopsis* mutant was strongly reduced by antimycin, but not by the *pgr5* mutation. Thus, as far as the AG is concerned, loss of PGR5 and antimycin poisoning are not equivalent. Actually, the effect of the *pgr5* mutation on the kinetics of PQ reduction in vitro did not fully mimic the effect of antimycin [19], and the exact role of PGR5 in the antimycin-sensitive electron cycle is still unresolved. Since the antimycin-sensitive component of AG luminescence was not reduced by the absence of PGR5, it is unlikely that this protein is an electron carrier that mediates directly the electron transfer from ferredoxin to PQ. This possibility was also excluded by Munekage et al. [19] who postulated that PGR5 could be involved in the stabilization or activation of the FQR complex. Seemingly, this function

is not essential for PQ reduction in the dark as measured by the AG TL band. This is consistent with the finding that the function of PGR5 is light-dependent [19]. Photosynthetic electron transport and P700 oxidation in *Arabidopsis* leaves were inhibited by the loss of PGR5 in high light, not in low light  $<100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . In contrast, antimycin was shown to inhibit leaf photosynthesis both in low light and in high light [17]. Thus, the antimycin-sensitive electron flux occurs at all light intensities while PGR5 seems to come into play in high light only, thus explaining why the *pgr5* mutation had no effect on the AG. The AG signal was also measured in *pgr5* mutant leaves preilluminated with white light and, again, no significant difference was found with WT leaves. Clearly, the exact physiological role of PGR5 remains to be elucidated.

The temperature at which the AG appeared was observed to vary substantially with the light conditions to which the leaves were exposed prior to the TL measurements. Prolonged dark adaptation or pre-exposure to far-red light led to an AG band peaking at a high temperature of about 45 °C. Pre-illumination of *Arabidopsis* leaves with white light or with a PSII-exciting light shifted the AG band towards lower temperatures, sometimes as low as 30 °C. An extreme case is provided by the luminescence emitted by *Synechocystis* after far-red light, which was restricted to a B band peaking at ca. 19 °C. In cyanobacteria, the photosynthetic electron transport chain and the respiratory chain are interconnected, with PQ as a common electron carrier [32,33]. Consequently, dark reduction of the PQ pool by soluble reductants is very fast in cyanobacterial cells relative to vascular plants [18]. This transfer could be fast enough to rapidly deplete the  $\text{S}_2/3 \text{ Q}_\text{B}$  states after switching off the far-red light, so that no light-emitting  $\text{S}_2/3 \text{ Q}_\text{B}^-$  centers could be formed during temperature elevation. Thus, one factor that determines the position of the AG band in the luminescence versus temperature plot is the rate of electron donation to PQ and the quinonic electron acceptors of PSII. In a previous study of several maize lines, we have found a correlation between the photoinduced downward shift of the AG band and the rate at which electrons are transferred from the stroma to  $\text{P700}^+$  after far-red light [13]. On the other hand, it is very unlikely that the  $T_\text{m}$  changes are due to changes in the PSII recombination properties. Indeed, it has been shown that the AG emission is limited by the electron back-transfer to the PQ pool, and not by the  $\text{S}_2/3 \text{ Q}_\text{B}^-$  recombination [5].

In *Arabidopsis*, there is clearly a continuum of  $T_\text{m}$  values which likely corresponds to different electron transfer rates from the stroma to the PQ pool and different thresholds of thermal activation of this transfer. We obtained various  $T_\text{m}$  values between 30 °C and 45 °C by playing with the pre-illumination conditions. These  $T_\text{m}$  changes were observed to occur rapidly (within minutes) following kinetics similar to those of the state 1–state 2 transitions and inducible by the same light conditions. Transition to state 2 by illumination with blue-green light was accompanied by a downshift to

almost 30 °C while transition to state 1 by far-red light was associated with an upward shift to 45 °C. In green algae, the state transitions are believed to be a system that regulates the linear versus cyclic electron fluxes [34]. Transition to state 2, which corresponds to the migration of LHCII and cytb6/f from the PSII-containing grana to the PSI-containing stroma lamellae [35], was correlated with a stimulation of cyclic electron transport around PSI [36–38]. In this study, transition to state 2 was also associated with an activation of cyclic electron transport as reflected by a downshifted AG band. However, the state transition itself was not responsible for the downward shift of the AG since high-light-acclimated leaves were still able to perform state transitions whereas the AG band was irreversibly ‘frozen’ at a low temperature of 32 °C. Moreover, the AG shifts were inhibited in *ndh Arabidopsis* mutants that are able to undergo normal state transitions. We can conclude that  $T_m$  is modulated by an independent phenomenon that is also involved in the state transitions and the NDH complex activity.

It is well known that state transitions involve phosphorylation/dephosphorylation of LHCII in response to changes in the redox state of the PQ pool [35]. Similarly, acclimation of plants to high light stress has been reported to induce sustained phosphorylation of thylakoid proteins [39], and a recent study has shown that the NDH complex can be phosphorylated in the light [40]. Consequently, protein phosphorylation is a phenomenon that seems to be shared by the state transitions, the function of the NDH complex and the AG shifts. Since the light-induced changes in  $T_m$  were strongly inhibited when the NDH complex was absent, phosphorylation of the NDH complex is an obvious candidate for the modulation of the AG peak temperature. The photoinduced change in  $T_m$  was less pronounced in tobacco leaves (~5 °C) relative to *Arabidopsis* (~15 °C), and this could be related to the minor role played by the NDH complex in the AG in the former species. Interestingly, the level of phosphorylation of the NDH complex has been correlated with the activity of the complex [40]. Therefore, the changes in activity and conformation of the NDH complex upon phosphorylation may decrease its thermal sensitivity and may induce electron flow through this complex at a lower temperature than in the non-phosphorylated state. In order to substantiate this hypothesis, we are currently analyzing by Western blottings whether low and high  $T_m$  values are associated with phosphorylation and dephosphorylation of NDH subunits, respectively.

It should be stressed that our results do not mean that state transitions are not involved in the regulation of cyclic electron transport. Our data suggest that state transitions are accompanied by a modification of the rate of electron donation from the stroma to the PQ pool and that this phenomenon could involve NDH phosphorylation. It is also important to remember that the AG signal results from a heat-induced electron flow mediated by the FQR and the NDH activities. Therefore, the AG is indicative of a part of

the electron cycle around PSI and it does not reflect all the complexity of the PSI-driven electron transport. For instance, our results indicate that the electron flux leading to the AG is activated by light. However, a number of previous studies have suggested that preillumination inhibits cyclic electron flow, with the fraction of PSI participating in the cyclic flow decreasing whereas that involved in the linear flow increases [41–43]. It was suggested that light-induced changes in the cyclic PSI activity could be a response to changes in ATP concentration. It is difficult to compare directly the results obtained in those previous works with our data because both the plant species investigated and the experimental conditions are very different (TL measurements in the dark vs. measurements in the light+diuron). However, it is clear that the cyclic PSI activity in vivo is subjected to complex regulatory processes and it is likely that the photoinduced shifts of the AG band reflects a part only of these regulations.

In conclusion, the AG signal originates from PQ-reducing electron fluxes that seem to follow the PSI-mediated cyclic electron transport routes. Consequently, the AG provides a new in vivo probe of the cyclic electron transfer pathways. An interesting aspect of the AG is that it can provide information on both the FQR and NDH activities. We have confirmed that the balance between NDH and FQR activity is variable, depending on the species. Moreover, the temperature ( $T_m$ ) at which the AG TL band occurred during temperature elevation was shown to depend on the light conditions to which the leaves were exposed prior to the TL analysis. These photoinduced changes in  $T_m$  reflect regulatory processes, possibly involving NDH phosphorylation, which affect the electron donation to the PQ pool. In *Arabidopsis* plants acclimated in the long term to high light at low temperature,  $T_m$  was maintained at a low value of about 30 °C which could not be reversed by far-red light, suggesting persistent phosphorylation of NDH. Interestingly, increased rate of electron donation from stromal reductants to PSI through the PQ pool was observed in leaves exposed to similar stress conditions [44]. We believe that the far-red light-induced AG luminescence will provide a new tool to study the bioenergetics of C3 plants, the cyclic PSI activity of which is difficult to assess in vivo by conventional methods. When measured in far-red light, cyclic electron transport around PSI is hardly detectable in C3 leaves, presumably because the redox state of the intersystem electron carriers controls the rate of PSI-driven cyclic electron flow in vivo [18]. In a previous study, it was necessary to use anaerobiosis as an experimental trick to reduce the PQ pool and to allow cyclic electron transport to be measured in far-red light [18]. Here, temperature elevation was used to induce reduction of the plastoquinone pool. Since the AG signal is emitted by PSII, this new method could be useful also in C4 plants, to provide information on cyclic electron transport around PSI in mesophyll cells without interference of the strong cyclic PSI activity in bundle sheath cells.

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