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REVIEW

Thermoluminescence in chloroplasts as an indicator of alterations in photosystem 2 reaction centre by biotic and abiotic stresses

A.N. MISRA*, F. DILNAWAZ, M. MISRA, and A.K. BISWAL

Department of Botany, Utkal University, Bhubaneswar-751004, India

Abstract

Thermoluminescence (TL) in green plants arises from charge recombination of charged molecules in the reaction centre (RC) of photosystem 2 (PS2) in chloroplasts. The TL technique is used for detection of alterations in the architecture of PS2 RCs. The donor side 'S-states' and the acceptor side quinone molecules (Q_A and Q_B) are involved the charge recombination processes of PS2. High temperature (70-75 °C) glow peaks are also used to detect non-photosynthetic peroxidation processes in thylakoid membranes. The TL peaks with their characteristic charge recombination can be utilised for the study of chloroplast development, ageing, chemical, biotic, and abiotic stress induced alterations in the PS2 RC and for the study of the primary photochemical events of photosynthesis. The technique has been used successfully in the characterisation of transgenic plants in the study of genetically engineered organisms.

Additional key words: heavy metals; mineral nutrition; peroxidation processes; photoinhibition; quinones; salt stress; thylakoids; senescence; transgenic plants; water stress.

Historical perspective: Thermally induced photon emission by a pre-irradiated chloroplast or thylakoid or by leaf samples in darkness is known as thermoluminescence (TL). This is a characteristic of solid states (semi-conductors) under thermally activated recombination of electrons and positive holes, that are generated by particle radiation or electromagnetic field at room or low temperature prior to their heating in dark (Chen and McKeever 1997). TL signals were first detected in dried chloroplast samples (Arnold and Sherwood 1957, Tollin and Calvin 1957). Photosynthetic systems in dried chloroplasts are supposed to be severely damaged. TL emission was also recorded in intact leaves and algal cells (Arnold and Sherwood 1957). Arnold (1966) proposed a model of recombination of free holes in PS1 and of free electrons from PS2 as the source of thermally induced luminescence from algae (*Chlorella*) cells in darkness. However, Arnold and Azzi (1968) refuted the role of free holes from PS1. Based on further evidence on the generation of charges in irradiated chloroplast, in the new model for TL charge recombination the positive and negative charge traps were proposed to reside within PS2 (Arnold and Azzi 1968). Further developments in the TL techniques and biophysical probing revealed that the charge pairs related to TL are generated in PS2. The TL signals arise as a reversal of the primary photochemical processes in PS2. Therefore they are also in some relation with the characteristics of fluorescence induction and emission (Ducruet 1999, Šetlíková *et al.* 1999).

There are reviews on thermoluminescence use in herbicide research (Horváth 1986) and general reviews on TL in chloroplasts or photosynthetic organisms (Sane and Rutherford 1986, Inoue 1996, Vass and Govindjee 1996). In the present review, we focus mainly on the use of TL in the studies of primary photochemical processes in chloroplasts. The use of TL technique in the studies of chloroplast development, and the impact of biotic and abiotic stresses on green plants are emphasised.

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*Fax: +91 (674) 581850, e-mail: misraan@yahoo.co.uk

Abbreviations: AG, afterglow; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; GMO, genetically modified organisms; Mo, membrane (per)-oxidation; PQ, plastoquinone; PS, photosystem; Q_A, primary quinone electron acceptor in photosystem 2 reaction centre; Q_B, secondary quinone electron acceptor in PS2 reaction centre; RC, reaction centre; TL, thermoluminescence; WOC, water oxidising complex.

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General principle for charge recombination in PS2: Thermally activated radiation recombination of positive and negative charges that are produced during irradiation induce primary charge separations in PS2. This gives rise to TL glow peaks. Samples are irradiated at room temperature to generate charge pairs within the PS2 RC and then rapidly cooled down with liquid nitrogen to trap these charge-separated states. The samples may be also irradiated during the cooling procedure. Subsequent warming (10-40 °C) leads to charge recombination and emission of luminescence glow peaks at characteristic temperatures. The emission temperature is characteristic of the charge pairs involved. This temperature is determined by the half-life (τ) of the recombining charges and the activation energy (E_A) for the charge recombinations (Chen and McKeever 1997). Using the equations for a first order kinetic equation, τ, E_A, TL intensity (I_{TL}), and absolute temperature (T) at which charge recombinations occur can be calculated taking the Randall-Wilkins model (Vass and Govindjee 1996). The charge recombination processes are multiple step phenomena in photosynthetic electron transport chain. However, approximating the multiple step charge recombination with a single step process, the kinetics of the photosynthetic TL process was proposed (Vass *et al.* 1981, DeVault *et al.* 1983, DeVault and Govindjee 1990, Ducruet and Miranda 1992, Vass and Govindjee 1996). The proposed equation is:

$$\begin{aligned} I_{TL}(T) &= -c\, dn/dT \\ &= cnk(T)/\beta \\ &= cn\, A \exp(-E_A/k_\beta T) \\ &= cn\,(k_\beta T/h) \exp(\Delta S/k_\beta) \exp(-E_A/k_\beta T) \end{aligned} \quad (1)$$

where c = proportionality factor, n = concentration of trapped charges, β = heating rate (kept usually at 20-30 °C for photosynthetic samples), k_β = Boltzmann's constant, A = frequency or pre-exponential factor, h = Planck's constant, S = entropy of activation.

Taking into account the thermodynamic parameters, the free energy of activation

$$\Delta G = E_A - T\Delta S \quad (2)$$

Hence the shape and the peak temperature of the TL curves are determined by a number of factors as shown in the above equations. Ducruet and Miranda (1992) developed a simulated curve fitting program using Eq. 1 and provided the prediction for E_A and half-life time (τ) of different charge pairs in PS2

$$\tau = ln\, 2/(AT)\,(E_A/k_\beta T)$$

The TL peak temperature (T) is one of the useful parameters for the TL glow curve analysis. However, the peak temperatures for specific charge recombination can differ significantly with the heating rate (DeVault *et al.* 1983) and the positioning of the thermocouple around the sample holder (Inoue 1996). The free energy of activation (ΔG) reflects the redox potential difference of the stabilising charge pairs and reflects the changes in the TL parameters (Vass and Govindjee 1996). Such energetic considerations can reflect a selective effect of treatments on either the donor side or the acceptor side of PS2. An up-shift in T indicates larger activation energy (E_A) of charge recombination. This shows a deeper trap on the donor side that also corresponds to the higher redox potential of the positively charged species but a lower redox potential on the acceptor side (Inoue 1996).

The stabilisation of charge-separated states occurs on the oxidising side of PS2. The immediate electron donor to $P680^+$ is Tyr 161 of the D1 polypeptide, which is denoted as Y_z. Y_z^+ is reduced by water oxidising Mn complex at a μs to ms time constant (Debus *et al.* 1988). The water oxidising complex (WOC) is postulated to exist in five redox states, denotes as S_0, S_1, S_2, S_3, and S_4 (Kok *et al.* 1970). Among them, S_0 and S_4 are the lowest and highest oxidation states, respectively (Fig. 1). Four electrons are sequentially extracted from the WOC. In the dark-adapted samples, the S-state cycle starts from the dark stable S_0 and S_1 states. S_2 and S_3 are stable for tens of seconds and S_4 is short lived in the dark. The electrons released by the WOC are trapped by the primary electron acceptor Q_A and subsequently transferred to the secondary quinone, Q_B (Fig. 1).

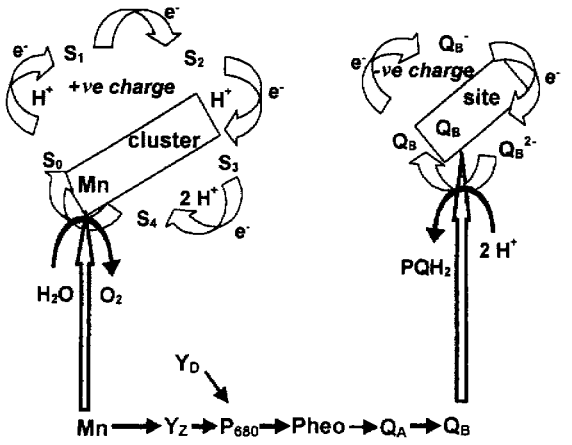

Fig. 1. Basic photochemical reactions in photosystem 2 reaction centre leading to water oxidation by Mn cluster and electron transport (*solid arrows in basal line* from Mn to Q_B) to the acceptor quinone molecules. S-state cycle or Kok cycle generates the positive charges by withdrawing e^- and H^+ from H_2O (*cycle in the left side top portions*). The negative charges are generated by the quinone reduction cycle forming semiquinol or quinol (*cycle in the right side top portions*). The positively charged Y_Z^+, Y_D^+, and P_{680}^+ molecules are generated during the electron transport process reducing the primary electron acceptor quinone Q_A to Q_A^-. Subsequently Q_A^- donates electron to Q_B forming semiquinol or quinol molecules.

TL glow peaks: TL glow curves are characterised by the temperature maxima of the emission band and are assigned to different charge recombination (Fig. 2). The

well-characterised TL glow peaks are those for A, AG, A_T, B, C, Mo, Q, Z, and Zv bands (Table 1). The TL bands Z and Zv arise at –169 °C and at –80 to –30 °C (Inoue 1996). These two bands are assigned to the charge recombination between charged pigment molecules. The Chl^+Chl^- charge recombination gives rise to Z-band and $P680^+Q_A^-$ charge recombination gives rise to Zv-band of the TL spectra. However, alterations in the PS2 RC is mainly studied through the changes in the TL bands A, AG, A_T, B, C, Mo, or Q. So a detailed characteristic of the charge pairs and the factors responsible for such glow peaks are explained. The influence of biotic and abiotic stress on the alterations of PS2 photochemistry deciphered through the TL characteristics are described.

A-band: A TL glow peak at –10 °C originates by photo-exciting photosynthetic samples by two flashes at room temperature, then cooling of samples to 77 K, and further continuous irradiation of the sample (Läufer *et al.* 1978, Inoue 1981, Tatake *et al.* 1981, Demeter *et al.* 1985). This band is designated as A-band. The charge recombination of $S_3Q_A^-$ is responsible for the origin of the A-band (Koike *et al.* 1986).

AG-band: An after glow (AG) or delayed luminescence rise induced by far-red radiation was first reported by Bertsch and Azzi (1965). This emission was reported only in intact systems such as intact chloroplasts (Hideg *et al.* 1991), leaves (Björn 1971), or protoplasts (Nakamoto *et al.* 1988), and could not be detected in isolated thylakoids or PS2 RC complexes. This AG glow peak at 45 °C could be resolved by slow heating of leaf samples (Miranda and Ducruet 1995a,b, Ducruet *et al.* 1997). The AG-band is suppressed by the PS2 electron transport inhibitor diuron (Miranda and Ducruet 1995a,b) and by the PS1 cyclic electron transport inhibitor antimycin or uncouplers (Björn 1971). This suggests that in addition to PS2, also the cyclic electron transport and/or the trans-thylakoid proton gradient are involved in the generation of AG glow peaks in leaves. Sundblad *et al.* (1988) assigned the AG glow peak primarily to the back reaction of $S_2/S_3Q_B^-$ charge recombination. The occurrence of AG peaks was also assigned to the presence of high concentrations of ATP and/or NADPH in plant cells (Inoue *et al.* 1976).

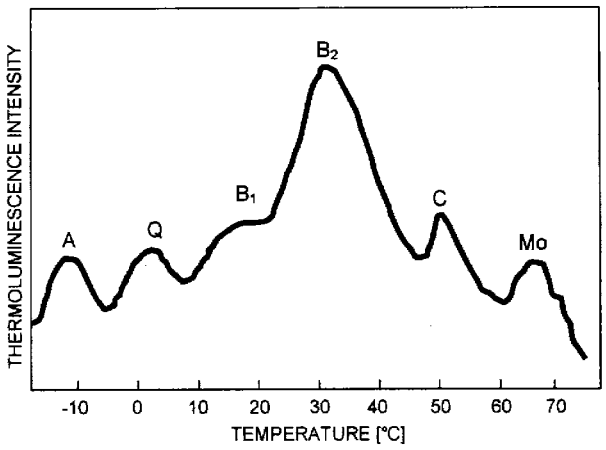

Fig. 2. Theoretical picture of thermoluminescence (TL) glow peaks of photosynthetic materials.

A_T-band: The Tris-washed PS2 particles show a TL glow peak similar to A-band (Inoue *et al.* 1977, Rózsa and Demeter 1982). Tris-washing depletes functional Mn clusters from PS2 particles. So the generation of S-states is impaired. Unlike the A-band, this band at –10 °C arises due to charge recombination of $His^+Q_A^-$ (Ono and Inoue 1991, Kramer *et al.* 1994). The positive charge is assigned to His195 and His190 residue of the D1 polypeptide (Kramer *et al.* 1994). This was confirmed by the impaired A_T-band in *Chlamydomonas reinhardtii* mutants substituted for the above His residues (Kramer *et al.* 1994).

Table 1. Nomenclature of TL bands used in Roman and Arabic forms. A new peak at 70 °C arising due to thylakoid membrane lipid peroxidation is designated as Mo [membrane (per) –oxidation] for distinguishing it from other high temperature bands.

TL band (Inoue 1996)	TL band (Tatake *et al.* 1981)	Temperature [°C]	Charge pairs	Mean life-time (τ, s) (Tatake *et al.* 1981)
A	II	-10	$S_3Q_A^-$	0.2
AG	-	+45	$S_2/S_3Q_B^-$	1.3
A_T	-	-10	$HisQ_A^-$	-
B_1	III	+20	$S_3Q_B^-$	-
B_2	IV	+30	$S_2Q_B^-$	29
C	V	+50	$Y_D^+ Q_A^-$	1 062
Mo	-	+70	Chl^+lipid peroxide$^-$	-
Q	-	+5	$S_2Q_A^-$	7.7
Z	Z	-169	Chl^+Chl^-	-
Zv	I	-80 to -30	$P680^+Q_A^-$	-

B-band: This is the best-characterised TL glow peak in photosynthetic materials. The B-band arises usually at around 30-35 °C. It is correlated to the water-oxidising complex of PS2 (Inoue 1976, Rózsa and Demeter 1982). Charge recombination between $S_2Q_B^-$ and $S_3Q_B^-$ generates the B-band (Rutherford *et al.* 1982). Exciting

the chloroplast or leaf samples with a series of brief flashes induced an oscillatory pattern of the intensity of B-band (Inoue *et al.* 1977, Rutherford *et al.* 1982, Horváth 1986). The analysis of oscillation pattern indicated the involvement of S_2- and S_3-states in the generation of B-band (Demeter and Vass 1984, Rutherford *et al.* 1984). The B-band glow peak characteristic is determined by the redox state of the quinone molecules. Both $S_2Q_B^-$ and $S_3Q_B^-$ charge re-combinations give rise to TL glow peaks of similar shape and position under physiological pH of 7.0-7.5 or above. However, the B-band is split into two components below pH 6.0. This phenomenon is due to the charge recombination $S_3Q_B^-$ and $S_2Q_B^-$ giving rise to B1- and B2-bands at 20 and 30 °C (Rutherford *et al.* 1985).

C-band: Photosynthetic materials show a TL glow peak approximately at 50 °C. The intensity of this band is enhanced by high concentrations of DCMU. So the participation of Q_A^- as the negative charge recombinant is suggested. The C-band is very stable and the lifetime of charge recombination at room temperature is probably 10 min (Demeter *et al.* 1984). This band was reported in leaves (Desai *et al.* 1975), chloroplasts (Rubin and Venediktov 1969), PS1 enriched particles (Sane *et al.* 1977), PS2 membrane particles (Sane and Rutherford 1986), and isolated D1/D2/Cyt b_{559} RC complex of PS2 (Rózsa *et al.* 1989). The C-band arises by the charge recombination of Q_A^- with S_0- and S_1-states (Demeter *et al.* 1984) or with Y_D^+ (Demeter *et al.* 1993, Krieger *et al.* 1993, Johnson *et al.* 1994). Although the flash induced oscillatory pattern of the C-band intensity showed the involvement of S_0- and S_1-states (Demeter *et al.* 1984, Demeter and Vass 1984), these two S-states do not provide any positive charge for recombination with Q_A^-. However, experiments with normal and Ca^{2+}-depleted PS2 suggests the involvement of Y_D^+ (tyrosine 160 residue of D2 polypeptide of PS2 RC) in the charge recombination with Q_A^- for the generation of C-band (Demeter *et al.* 1993, Krieger *et al.* 1993, Johnson *et al.* 1994).

M_0-band (TL 75 °C band): A TL band at 70-75 °C arises due to the peroxidation of thylakoid membrane lipids during heating processes (Hideg and Vass 1993). We propose to name it M_0-band. Similar glow peaks in spinach chloroplasts were reported by Desai *et al.* (1982). According to these authors, origin of this curve is due to chemiluminescence of Chl resulting in destruction of thylakoid membranes. However, it is now accepted that this band preferably originates by radical induced processes in chloroplasts (Hideg and Vass 1993).

Q-band: Treatment of chloroplasts or leaves with PS2 herbicides or DCMU inhibits electron transfer from Q_A to Q_B, abolishes the B-band, and stabilises electrons on Q_A with a concomitant appearance of a new TL band at 0 to 10 °C (Rubin and Venediktov 1969). Demeter and Vass (1984) assigned the charge recombination of $S_2Q_A^-$ for the generation of new TL band designated as the Q-band. This band is used for the analysis of stabilisation of the S_2-state. The Q-band showed *in vivo* a constant peak temperature throughout the physiological pH. Q_A is probably deeply buried under a deeper trap in the D_2-polypeptide of the PS2 RC and so is resistant to pH changes *in vivo*. Also the transition from S_1 to S_2 does not involve the release of protons (Fig. 1) and so no pH dependence is expected (Vass and Inoue 1986). On the contrary, DCMU-poisoned isolated thylakoid membranes showed a TL peak at 11 °C at pH 5.3 and 7.5. The TL peak of the above sample was reported to shift to 28 °C with alkali treatment at pH 8.4-9.0 (Vass and Inoue 1986). Treatment of thylakoids with dithionite, which completely reduces Q_B, showed similar pH-induced changes at TL peak temperature of the Q-band. However, partial proteolysis of thylakoid membranes by trypsin showed an increase in T by acidification (pH = 5.3) or alkalisation (pH = 8.4). This anomalous response was interpreted as an indirect effect of higher pH on the donor side of PS2, and the possibility of direct effect of proton on Q_A or S_2 was meagre (Vass and Inoue 1986).

Developmental aspects:

Greening: Major TL bands are missing in dark grown leaves. Greening of leaves under widely spaced intermittent irradiation or in algae grown in Mn-deficient medium also showed no major TL bands. However, the TL bands appeared when the water oxidising systems were activated in the above systems by continuous irradiation or closely spaced flashes. These studies suggest that water oxidising complex is involved in the generation of TL as a positive charge reservoir (Inoue *et al.* 1976, Sane *et al.* 1977, Hideg and Vass 1993). Misra and Misra (1987) showed age dependent developmental changes of chloroplasts in greening wheat leaves. Further studies on the developmental gradient of chloroplasts along the wheat leaf blade showed that the TL intensities of Q and B band increased from base to apex (Misra *et al.* 1998b). This suggests an increase in accumulation of both Q_A and Q_B species along with the S states forming the charge pairs for the respective TL bands.

Senescence: Chloroplast senescence can be monitored during natural ageing of leaves under irradiation or accelerated ageing of the leaves under continuous darkness (Misra and Biswal 1980, 1981, 1982). Senescence can also be accelerated by stress factors (Misra and Misra 1986, Misra *et al.* 1995, 1997a,b, 1998a, 1999). Chloroplast ageing both in light and darkness induced a decrease in normal TL bands (Joshi *et al.* 1993). The decrease in TL band intensities could be due to a decrease in the number of charge pairs or decrease in the number of active PS2 RCs during senescence. A down-shift in peak temperature of the B-band was reported during chloroplast senescence in wheat leaves (Joshi *et al.* 1993). Although the authors interpreted their data on the basis of D1-polypeptide modification in dark, this could be due to the changes in

the redox status of the charge pairs during leaf senescence. The changes in –12 and +10 °C bands were assigned to the changes in $S_2Q_A^-$ and $S_3Q_A^-$, and thermodynamic calculations suggested a blockage in the $S_2 \rightarrow S_3$ transition (Joshi *et al.* 1993). This interpretation was based on the fact that damage of Q_A molecules is less probable because they are buried in the D2 polypeptide of the RC of PS2.

Abiotic stress:

Mineral nutrition: Solntsev *et al.* (1998) studied the effects of N, P, K, or their mixtures under soil and sand culture on three cultivars of wheat. First leaf of 10-d-old seedlings of cvs. Mironovskaya and Markiz were taken as leaf samples. The samples were cooled to –30 °C after red irradiation at room temperature. The frozen samples were then irradiated with incandescent lamp at –30 °C for 3 min. The A-band which arises due to S_4/S_3-Q_A^- charge recombination, appeared at –13 to –18 °C in plants cultured with the mineral salts. However, depletion of minerals shifted the band to a higher temperature at –8 °C. The shift in the T_{max} could be due to the accumulation of S_4 states in the leaves supplied with mineral salts.

Salt stress: Salinity affects PS2 activity and all TL glow peak intensities. Analysis of the B and Q TL glow peaks suggested that both $S_{2/3}Q_B^-$ and $S_2Q_A^-$ charge recombination was differentially affected by salt stress (Sahu *et al.* 1998, Misra *et al.* 1999). The B-band intensity decreased faster than the Q-band, and after few days the salt stressed seedlings showed also a decrease in the Q-band intensity. Recent studies by our group showed that these changes are dose and time dependent (Misra *et al.*, unpublished).

Water stress: The far-red irradiation induced B- and AG-bands are more sensitive to drought stress than the flash induced B-band. So, this is a sensitive indicator for stress studies (Ducruet and Vavilin 1999, Janda *et al.* 1999). Drought stress had a reversal effect on far-red induced downshift in the peak temperature maxima of B-band and induced a decrease in the AG-band intensity. This is explained by the induction of proton leakiness of stressed thylakoids and/or by the state of the reducing pool in chloroplasts.

Heavy metal toxicity: Heavy metals generally affect PS2 photochemistry (Demeter and Govindjee 1989). Treatment of isolated thylakoids with Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} affects the B-band, but has no or little effect on the Q band (Mohanty *et al.* 1989). These results suggest that these heavy metal ions act at the Q_B site.

Inert gases: Inert gases such as N_2, He, Ar, Xe, and CO_2 (creating anoxic environment in the leaf) decrease O_2 concentration in leaf environment. This leads to the decrease in TL intensity of B- and C-bands, but slightly affects the band A (Solntsev *et al.* 1998). The C band increased in pure O_2, but the A- and B-bands remained constant as those of the control leaf. This change in TL glow peaks occurs when pure air is replaced by the above-mentioned gases during heating the sample and not during irradiation.

Halogens: Effect of I^- and Cl^- was compared using KCl or KI on isolated chloroplasts and leaves (Solntsev *et al.* 1995). High concentrations of KI induced quenching of TL bands. The I^- ion may donate electrons to PS2 and can associate with the Cl^- binding domain of the RC polypeptides (Homann *et al.* 1986). Probably I^- donates electrons to S-states bringing down (quenching) the TL peaks. KCl did not show such effects. So the TL quenching effect in the presence of KI may be used to determine the permeability of different photosynthetic membranes to I^-. This indicates a possible participation of monovalent ions in the regulation of the primary reactions linked to PS2.

Spectral composition: The leaves irradiated with 625 and 725 nm radiation during cooling showed similar TL spectral composition of B- and C-bands (Miranda and Ducruet 1995a). However, the A-band was missing in leaf samples irradiated with monochromatic radiation of 725 nm. This could be due to the absence of S_4 states in the water-splitting complex of PS2, as the 725 nm radiation is absorbed predominantly by PS1. Also leaves cooled without irradiation or on exposure to "white light" have an identical form of TL bands. Miranda and Ducruet (1995b) showed that leaves irradiated with far-red radiation at low but non-freezing temperatures emit a TL band peaking at around 40-45 °C together with the B-band peaking below 30 °C. This band at 40-45 °C is designated as AG-band. Increasing the duration of the far-red irradiation caused a progressive downshift of the B-band (Miranda and Ducruet 1995a), which was assigned to the destabilisation of S-states by progressive lumen acidification (Solntsev 1995).

Photoinhibition: Green plants exposed to high irradiance under oxygenic conditions are subjected to photoinhibition of PS2. The decrease in B-band is positively correlated to the degradation of D1-polypeptide in PS2 RCs (Misra *et al.* 1997a, 1998a,b). However, the oscillatory pattern of the TL peak showed similar rhythmicity as that in control leaves, suggesting that the S-states were unaffected by photoinhibition. TL band C arising at 50 °C is resistant to photoinhibition change at room temperature (Misra *et al.* 1997a). The acceptor side of PS2 may be more susceptible to photoinhibition than the donor side. However, S-state stabilisation in a mutant of *Synechocystis* was not directly related to the sensitivity to high irradiance (Constant *et al.* 1996).

Isolated thylakoid membranes subjected to stress irradiance showed a parallel decrease in the Q- and B-bands, suggesting both the primary and secondary quinones are affected in this system (Vass *et al.* 1988).

Algae cells recovering from photoinhibition under weak irradiance showed a redox change at the peak temperature of B-band (Ohad *et al.* 1990). These redox changes in B-band were interpreted as a reversible

conformational change in the Q_B binding pocket of the D1-polypeptide in PS2 RC (Ohad *et al.* 1988).

High temperature affected the TL yield of pothos leaf discs (Joshi *et al.* 1995). However, although the TL values decreased above 42 °C, a large number of affected PS2 units retained a functional water-oxidising complex at the donor side. Misra *et al.* (1997a, 1998) reported that the Q- and B-bands are affected by photoinhibition at chilling, room, and high temperatures, but the C-band is resistant to temperature induced accelerated photoinhibition in pothos and spinach leaf discs. When maize and wheat leaves were heat-stressed for 5 min, the AG-band (glow peak temperature around 45 °C) decreased progressively with an increase in the incubation temperature and was completely suppressed when the leaves were incubated above 35 °C (Janda *et al.* 1999). An increase in membrane leakiness and grana de-stacking are the probable cause for the heat-induced changes in the AG-band.

Freezing/frost damage: Freezing at –10 °C had no effect on the T_{max} of TL bands in spinach, pea, and wheat leaves (Janda *et al.* 1999). However, the B-band intensity decreased significantly. There was a downshifting of the B-band to 20-22 °C in maize and vines after short term freezing. This change in the B-band was not detected in cell-free thylakoids and so it may be a freezing artefact. However, the frost treatments partially reversed the downshift of B-band induced by far-red irradiation (Miranda and Ducruet 1995a). This can be explained as a destabilisation of S-states by progressive acidification of thylakoid lumen (Miranda and Ducruet 1995b). The action was similar to that caused by uncoupler and was ascribed to freezing induced damage to the thylakoid membrane (Thomashow 1998), leading to the collapse of the proton gradient (Janda *et al.* 1999).

Several plant species showed a sensitivity of the AG peak to freezing (Nakamoto *et al.* 1988). Compared to the B-band, the AG-band was more sensitive to freezing (Janda *et al.* 1999). There was usually a sudden drop in the AG-band after 2 min incubation of wheat and maize leaves at –10 °C. The temperature range within which the AG-band is suddenly lost was within 1 °C. This critical temperature depends on several factors such as frost hardening and genetic composition. Frozen wheat samples showed a week AG-band, but it was completely suppressed in frost sensitive maize plant (Janda *et al.* 1999).

Biotic stress:

Viral infections of host plants decrease the photochemical efficiency of PS2 (Balachandran *et al.* 1994). The degree of PS2 inhibition depends both on growing conditions of plant and on the viral strain. PS2 remains functional to a great extent when infected with viruses that do not induce external symptoms in their host (asymptomatic). However, a drastic decrease in the PS2 activity was reported in host plants infected with viruses that induce symptoms (van Kooten *et al.* 1990). The exact mechanism of action of viral infection on PS2 is still unknown.

The TL glow peaks of thylakoids from *Nicotiana benthamiana* plants infected with mottle viruses showed a shift in B-band towards a higher temperature indicating changes in the redox state of the charge pairs (Rahoutei *et al.* 1999). This shift suggests a shift of $S_3(S_2)Q_B^-$ to $S_2Q_B^-$. Further progress of viral infection generates a new band at 70 °C with a simultaneous decrease in the B-band intensity. During this period visible symptoms appear.

Hypersensitive reactions: Elicitor-induced hypersensitive reactions generally induce peroxidative reactions in the host cells. Stallaert *et al.* (1995) reported that the elicitor cryptogein induced a dominant high temperature TL glow peak in tobacco leaves. These authors concluded that appearance of the high temperature (70-75 °C) band represented the peroxidative breakdown of thylakoid membrane lipids and can be used as a stress indicator for biotic stress.

Genetically modified organisms (GMO) or Mutant studies: Recent advances in genetic engineering techniques to develop herbicide resistant, stress resistant, photo-autotrophic and photosynthetically efficient mutants, or genetically modified organisms (GMOs) open a new vista in photosynthetic research. Challenges in the field of productivity and crop improvement can be combated through the use of non-invasive and quicker techniques such as TL studies in GMOs. PS1 and PS2 mutants led to the identification of PS2 as the origin of most of the TL bands (Lurie and Bertsch 1974, Ichikawa *et al.* 1975, Sane *et al.* 1977, Debus *et al.* 1988, Minagawa *et al.* 1999). The site of genetic modification in PS2 through genetic engineering techniques can be detected by this technique (Lurie and Bertsch 1974, Ichikawa *et al.* 1975, Debus *et al.* 1988, Minagawa *et al.* 1999).

Future directions and perspectives: TL technique provides a powerful non-invasive technique for deciphering the subtle changes in the PS2 RC. However, the instrument is yet to be used in a versatile manner, as the devices available are still not handy and portable. So most of the work done are bench top experiments and not on the spot verification in the field conditions. Use of alternative coolants and methods to analyse only the desirable TL peaks, which can be generated by a temperature range not detrimental to the field grown plants, is highly desirable. Besides improvement on the instrumentation parts, further characterisation of new and useful TL peaks will be certainly a desirable effort for utilisation of this tool for photosynthetic and field research.

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