Chapter 6
Thermoluminescence: A Tool to Study Ecophysiology of Green Plants

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1 Introduction

Thermally induced light emission in physical chemical or biological systems is known as thermoluminescence or TL (Demeter and Govindjee 1989; Misra and Ramaswamy 2001; Misra et al. 2001a, b, 2012; Ducruet 2003; Maslenkova 2010; Sane et al. 2012; Misra 2013). This phenomena is the characteristic of a solid state or semi-conductor, in which thermally activated recombination of electrons with positive holes is generated by particle or electromagnetic radiation at room or low temperature prior to their heating in dark (Randall and Wilkins 1945; Demeter and Govindjee 1989). Luminescence occurs in materials absorbing light. Light energy absorbed by a system induces photochemical reactions and transduces light/photon energy to kinetic and/or chemical energy. Excess light energy that is not utilized by photochemical processes are emitted back or dissipated in various forms of luminescence *viz.* fluorescence, phosphorescence, delayed luminescence, chemiluminescence and thermoluminescence (Misra et al. 2001a, b, 2012). The time course of the emission lifetime of this luminescence is given in Table 6.1. These are the phenomena of de-excitation of any photo-excited materials. The quantum yield of the de-excited system is less than the excited state, due to internal conversion of energy and/or heat dissipation. Thermoluminescence (TL) is the characteristic of a system that emits light at a characteristic temperature due to the chemiluminescence properties, radical pair states, or electron hole pairs (Misra et al. 2001a, b, 2012; Ducruet 2003). The biophysical analysis of the charge recombination shows that the phenomenon in darkness is the reversal of the primary photochemical processes in PS II (Misra et al. 2001a, b; Sane 2004). In the present chapter, the practical use of

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Table 6.1 The lifetime of different luminescence from an excited material

Luminescence	Temperature dependent or independent	Half life of emission after excitation (τ_c)
Fluorescence	Independent	$<10^{-8}$ s
Phosphorescence	Dependent	$>10^{-8}$ s
Delayed luminescence	Independent	Minutes $< \tau_c <$ years
Thermoluminescence	Dependent	Minutes $< \tau_c < 4.6 \times 10^9$ years

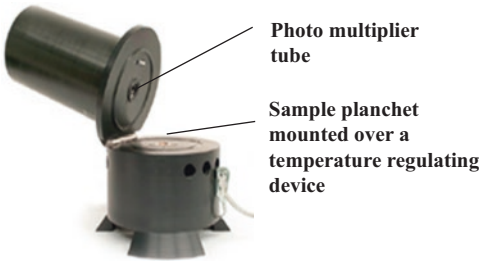

Fig. 6.1 A thermoluminiscence apparatus set up

TL for the study of the assessment of environmental impact on the changes in the primary photochemical processes of PSII is explained.

2 Instrumentation

TL measurement is done usually with an assembly of dark chamber, a copper planchet with a temperature sensor, a manual or peltier cooling/heating device, red-sensitive photo multiplier tube, signal amplifier, and a X-Y recorder or data acquisition instrument/computer (Tatake et al. 1971; Ducruet and Miranda 1992; Zeinalov and Maslenkova 1996; Bhatnagar et al. 2002; Ducruet 2003; Gilbert et al. 2004b). The samples of photosynthetic materials are photo-excited by several (8–10 nos.) flashes of short (5 ms) duration and cooled either to liquid nitrogen temperature in order to keep the charge particles in a physically separated state. Depending on the experimental requirement, one can also cool the samples to sub Zero temperatures. Then the samples are heated in a gradual, slow and linear heating mode to induce charge recombination, giving rise to a set of different TL emission bands as a result of recombination of different charge pairs at a particular temperature (Misra et al. 2001a, b, 2012). A picture of the TL set-up is shown in Fig. 6.1. These characteristic TL bands are used in the study of various biotic and abiotic stress factors in green plants (Misra et al. 2012). Photosynthetic materials are directly placed on the sample holder and excited by (i) continuous light during freezing or (ii) excited by flash(s) of saturating pulse, series, or (iii) excited by combining

(i) with (ii) at a particular temperature prior to flash freezing the sample. (Ducruet and Vass 2009; Sane et al. 2012). There are several TL apparatus commercially available by Photon Systems Instruments (Brno, Czech Republic), which provides spectral deconvolution programs (Ducruet and Miranda 1992).

3 Thermoluminescence Glow Peaks

Photosynthetic materials, such as isolated photosystem II (PS II) pigment-protein complexes, thylakoid membranes, chloroplasts, cyanobacteria, algae and green leaves illuminated with a saturating flash of light, induce charge separation in PS II. The TL measurement is done by photoexcitation of the leaf sample, then cooling it at liquid nitrogen or to a low temperature, followed by heating in dark and recording the photo-emittance during heating (Tatake et al. 1971; Zeinalov and Maslenkova 1996; Misra et al. 2001a, b, 2012; Ducruet 2003; Gilbert et al. 2004a, b; Bhagwat and Bhattacharjee 2005; Ducruet and Vass 2009). But recent commercial instruments use a peltier cooling and heating system. The TL emission is then measured with a sensitive photomultiplier. The emission around 730 nm vs. temperature is plotted in a graph sheet. Arnold and Azzi (1968) showed the occurrence TL glow peaks between −40 °C and +50 °C in photosynthetic materials. In pre-irradiated photosynthetic materials (pigment protein complexes, thylakoid membranes, intact chloroplasts or green leaves) TL glow peaks arise in darkness (Misra and Ramaswamy 2001; Misra et al. 2001a, b, 2012; Misra 2013). The separated charge pairs recombine and emit photon. Saturating and sequential short pulses (in ms scale) light generates S0, S1, S2, and S3 states in the water oxidizing Mn cluster of PS II. The S0 and S1 states remain stable during darkness. Upon illumination, these states are photo-converted and the S^o and S^1 states are distributed in 25% and 75% approximately in the photosynthetic materials. In leaves, approximately 40% of Q_B is reduced (Rutherford et al. 1984a) and the Q_B^-/Q_B ratio oscillates with a periodicity of 2 flashes. When leaf photosynthesis is inhibited or the electron transfer from Q_A to Q_B in PS II is blocked, only Q_A^- charge accumulates. The recombination of charges and holes at a particular temperature and emission of photon is designated by specific nomenclatures as shown in Table 6.2. This charge recombination of Q_A^- and Q_B^- with S2/S3 results in Q band and B band, respectively, at around 5 °C and at 20–35 °C. The B- band is the major TL band observed in any photosynthetic material studied so far (Fig. 6.2). The charge recombination of S1/S2 states with Q_A^- is less stable than that of B band and recombines quicker than Q_B^-. These recombinations are very sensitive to redox changes in the charge pairs (Misra and Ramaswamy 2001; Misra et al. 2001a, b, 2012; Misra 2013). Thus, any change in the stable environment of PS II can be measured by the changes in TL glow peaks. As it also oscillates with each flash number, the redox state of Mn cluster can be titrated with TL measurements (Misra et al. 2001a, b, 2012; Misra 2013).

Table 6.2 Characteristic thermoluminescence (TL) bands from photosynthetic materials. These bands are reported in PSII particles, thylakoid membranes, chloroplasts, cyanobacteria, algae and green leaves

TL band	Temperature	Charge recombination	References
Very low temperature TL peaks (LTL)	−200 to 250 °C	Chlorophyll aggregates	Sane et al. (2012)
Z	−160 °C	$Chl^+ Chl^-$	Misra et al. (2001a, b, 2012)
Zv (variable)	−80 to 30 °C	$P680^+ Q_A^-$	Sane et al. (2012)
A	−15 °C	$Tyr Z^+ QB^-$	Misra et al. (2001a, b, 2012)
AT	−10 °C	$S_3Q_A^-$	Tatake et al. (1971), Inoue et al. (1977), Rosza and Demeter (1982), Demeter et al. (1985) and Homann (1999)
Q	+5 °C	$S_2Q_A^-$	Misra et al. (2001a, b, 2012)
B1	+20 °C	$S_3Q_B^-$	Inoue (1976), Joliot and Joliot (1980), Vass et al. (1981), Rutherford et al. (1982, 1984b), Demeter and Sallai (1986) and Miranda and Ducruet (1995b)
B2	+30 °C	$S_2Q_B^-$	
C	+50 °C	$TyrD^+Q_A^-$	Misra et al. (2001a, b, 2012)
AG	+40 to 50 °C	$S_2/S_3Q_B^-$	Bertsch and Azzi (1965), Bjorn (1971), Inoue (1996), Nakamoto et al. (1988), Sundblad et al. (1988), Hideg et al. (1991), Johnson et al. (1994) and Miranda and Ducruet (1995a, b)
High temperature TL peaks (HTL)	50 to 160 °C	Oxidative chemi-luminescence	Venediktov et al. (1989), Vavilin et al. (1991), Merzlyak et al. (1992), Hideg and Vass (1993), Stallaert et al. (1995), Misra et al. (1997), Marder et al. (1998), Vavilin and Ducruet (1998), Ducruet and Vavilin (1999), Havaux and Niyogi (1999), Skotnica et al. (1999), Havaux and Niyogi (1999) and Ducruet and Vavilin (1999)

For more details see Sane and Rutherford (1986), Inoue (1996), Misra and Ramaswamy (2001), Misra et al. (2001a, b, 2012), Sane et al. (2012) and Misra (2013)

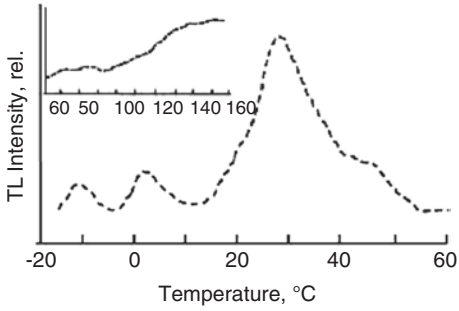

Fig. 6.2 Typical thermoluminescence (TL) peaks observed in a green leaf with a linear heating rate of ≤20 °C/s in darkness. (From Misra 2013)

4 Stress Induced Changes in TL Glow Peaks

The TL glow peaks as depicted in Table 6.2 clearly show that TL is a useful tool for the study of PS II electron transfer, both at the donor and acceptor sides (Misra and Ramaswamy 2001; Misra et al. 2001a, b, 2012; Misra 2013). Extensive reports are available to suggest that biotic and abiotic stress bring about a qualitative and quantitative change in the TL peak temperature and intensity. The changes in the TL characteristics and the environmental factors affecting it are summarized in Table 6.3.

Table 6.3 Changes in TL glow peaks of photosynthetic materials induced by developmental and stress (biotic and abiotic) responses (Misra and Ramaswamy 2001; Misra et al. 2001a; b, 2012; Misra 2013)

Environmental stress and plastid development	Changes in TL glow peaks	References
Plastid development		
Etiolated leaf	Major TL peaks missing due to a lack of functional pigment-protein complexes associated with PS II and do not develop Mn cluster	Inoue (1996), Sane et al. (1977), Misra et al. (1998a, b, c) and Dilnawaz et al. (2000)
Greening leaf	Q-band and B band intensity increases gradually from base to apex of that wheat leaves greening under continuous illumination	Misra et al. (1998b)
	Leaf greening under intermittent illumination leaves does not show the TL bands, as these plastids do not develop Mn cluster properly	Inoue (1996) and Sane et al. (1977)
Aging and senescence of leaf	Decrease in Q and B-band.	Biswal et al. (2001)
	The titre shows a gradual decrease in quinone pool and a block in electron flow between QA to QB.	
Genetic modification	Origin of Tl glow peaks	Farineau (1993) and Homann (1999)
Biotic stress		
Pathogen (viral) infection	Decreased B-band intensity and higher peak temperature.	Stallaert et al. (1995) and Rahoutei et al. (1999)
	A new TL peak at 70 °C	
Abiotic stress		
Salinity	Affects Q-band and B-band in a dose and duration dependent manner. B-band comparatively more affected.	Misra et al. (1998c), Sahu et al. (1998, 1999), Biswal et al. (2001) and Zurita et al. (2005)
	Back flow of electrons in PS II.	
Water/drought	Temperature shift in the TL glow peaks due to redox shift in the charge pairs	Ducruet and Vavilin (1999), Janda et al. (1999) and Misra et al. (2002)

(continued)

Table 6.3 (continued)

Environmental stress and plastid development	Changes in TL glow peaks	References
Anoxia	Reduces the B and C band intensities	Soltnev et al. (1999)
Mineral supplement (N, P, K)	A band shift from nearly −13 °C to 8 °C	Soltnev et al. (1999)
Heat/high temperature	Decrease in Q-band and B-band intensity	Misra et al. (1997, 1998a)
Photoinhibition	B-band affected with less effect on C-band	Misra et al. (1997, 1998a)
State transition	B-band affected	Bernat et al. (2018)
Heavy metal (Cu, Ni or Zc)	B-band affected	Mohanty et al. (1989)
Inert gas (N, He, Ar or Xe)	Reduces the B and C band intensities	Soltnev et al. (1999)

5 Future Perspective

Environmental and developmental changes affect the photosynthetic machinery (Joshi et al. 2013). Thermoluminescence is a non-invasive method and can give insight into the qualitative and quantitative changes in the Q_A and Q_B environment of PS II and thus give an insight to the donor/acceptor side structure and function, and also the oxidative state of thylakoid membranes (Misra and Ramaswamy 2001; Misra et al. 2001a, b, 2012; Misra 2013). Recently, TL signals have been used as 'sensors' for the study of photosynthetic materials (Zhang et al. 2007). TL technique gives a wide array of information about the redox state of electron donors, acceptors and charge accumulation in PS II of green leaves, and TL studies have an extensive and wide use in eco-physiological and stress studies in photosynthesis, as well as in agriculture.

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