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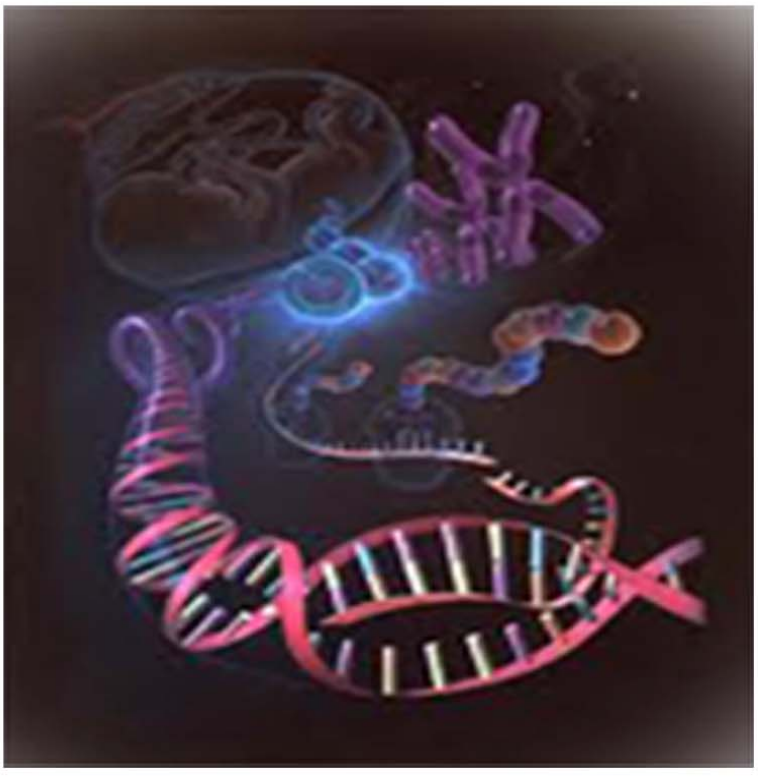

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Review Article

THERMOLUMINESCENCE IN PLANTS: CONCEPT AND APPLICATION

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Luminescence that is thermally induced is known as thermoluminescence (TL). TL is a characteristic of solid states which arise by thermally activated recombination of electrons and positive holes. In green leaves, the recombination of the positive and negative charge traps within PS II gives rise to TL emission. The technique is used for the assessment of pertubations in the photosystem induced by stress or genetic modification of the plants. In this review the concepts about this technique and its use is explained.

Keywords: Thermoluminescence, Abiotic, Biotic, Stress, Photosynthesis, Thylakoid membrane, GM plant

INTRODUCTION

Light energy from sun is absorbed by green plants through photosynthesis which sustains life on earth. Each photon absorbed by a living system elicits photochemical reactions, convert photon energy into kinetic or chemical energy. Excess of that utilized for photochemical process is emitted as luminescence. Luminescence occurs in almost all the materials absorbing photon energy and is a phenomenon of light emitting process through fluorescence, phosphorescence, delayed luminescence, chemiluminescence and thermoluminescence. Light is emitted back from a molecule when a photon or chomo-excited molecule is deexcited to its ground state (Misra *et al.,* 2012). During this deexcitation process, radiationless internal conversion and heat dissipation occurs, which reduces the quantum yield of light emission. The luminescence that is thermally induced is known as thermoluminescence (TL) and describes the emission of light at characteristic temperatures from samples containing chemicals with chemiluminescence properties, radical pair states or electron hole pairs (Ducruet, 2003; Misra *et al*., 2001 and 2012). TL has been used initially in geology, archeological dating and radiation dosimetry. The theory of charge recombination

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in these processes was first worked out for such minerals (Randall and Wilkins, 1945). This is a characteristic of the solid states (semi-conductors) which arise 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). In green leaves, thermally induced photon emission by a pre-irradiated chloroplast or thylakoid or by leaf samples in darkness is reported (Misra *et al.*, 2001; Misra and Ramaswamy, 2001). Studies by Arnold and Azzi (1968) established the involvement of the recombination of the positive and negative charge traps within Photosystem II (PS II) in the appearance of TL peaks in plants between the temperature range of –40 °C to +50 °C.

INSTRUMENTATION

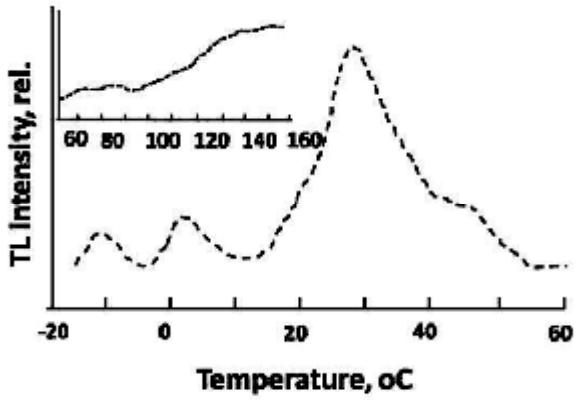
A set of different bands in TL emission curves appear as a result of recombination of different charge pairs (Misra *et al.,* 2001, 2012). Even small changes in the redox properties of radical pairs affect the intensity and the peak position of TL bands. This complexity of information of TL emission curves can be used for selective monitoring of the effects of various biotic and abiotic stress factors on plants (Misra *et al.,* 2012). TL bands at high-temperatures above 60°C appears as a result of accumulation of lipid peroxides (Misra *et al*., 2001) and is used as a simple and efficient tool to monitor oxidative stress in leaves (Havaux, 2003). The progress in the development of the TL techniques and biophysical analysis, of the charge recombinations, revealed that the phenomenon arise as a result of reversal of the primary photochemical processes in PS II (Misra *et al.*, 2001a, b; Sane, 2004). In the present review, the use of TL in studies of the assessment of the changes in the primary photochemical processes of PSII is explained. The impact of environmental factors affecting chloroplasts are also discussed.

The TL measurement is done by photo-excitation of the leaf sample, then cooling it at liquid nitrogen or to a low temperature, followed by heating in dark and recording the phot-emittance during heating (Misra *et al.,* 2001a, b; Tatake *et al*., 1971; Manche, 1979; Zeinalov and Maslenkova, 1996; Gilbert *et al.*, 2004; Ducruet, 2003, Ducruet and Vaas, 2009). In recent years, a peltier cooling and heating system is used in the instrument. The ultra weak TL emission is then measured with a sensitive photomultiplier. The emission around 730 nm in leaves is measured against the rise in temperature . The TL glow curve or luminescence bands are shown in Figure 1. A detailed analysis on the effect of freezing on the photon emission shows that there can be artifacts generated during freezing due to ice crystal formation in the intracellular space in the leaves (Homann, 1999). However, this is also system specific and should be checked before the analysis of the TL signals.

CHARGE RECOMBINATIONS

The green leaf when illuminated with a saturating flash of light, induces charge separation in PS II. This charge separated states recombine to stabilize the charge pairs, and is able to emit photon. Saturating light creates an equilibration of S_0, S_1, S_2, and S_3 states. The states S_0 and S_1 remain stable during dark. Upon illumination S_2 and S_3 are converted to S_1, resulting in a 1/4 S_0 3/4 S_1 distribution. 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

Figure 1: The photosynthetic TL peaks that at different temperatures in a unstressed green leaf, The inset is for high temperatures, The peak height and temperature can vary with treatments



flashes. When a leaf photosynthesis is inhibited and electron transfer from Q_A to Q_B is blocked, the electron is stored in the primary electron acceptor of PS II, as Q_A^-. This is a less stabilized state compared to that of B band due to recombination of Q_B^- and so recombines quicker than the later to produces a Q-band peaking at a lower temperatures around 5 °C than the B band at 20-35 °C, upon recombination with S_2/S_3. The oxidized Tyrosine D (D^+) also recombines at a slow rate with Q_A^- giving rise to a C-band at about 55 °C (Johnson *et al*., 1994). The functional electron donor to the PSII centre is: $Z^+P680^*/TyrZ^{++}P_{680}^-$. A fast rereduction of Tyrosine Z^+ by S states do not yield any TL glow peak. But, when the oxygen-evolving complex is damaged, which leads to a low temperature band due to recombination of $Z^+Q_B^-$ at about –15°C. This band is known as A band. Other TL bands exist at lower temperatures which are not well characterized. Although PSII can be totally destroyed at about 60°C, a number of chlorophyll TL bands can be observed at higher temperatures. They correspond to a heat-enhanced chemiluminescence from molecular species generated by radical forms of oxygen, such as lipid peroxides, which accumulate in stress situations. Despite the fact that the mechanisms of high-temperature TL emission (HTL) is completely different from photosynthetic TL, recorded through a single temperature scan from 0°C to 160°C on the same leaf disc, both the photosynthesis TL bands and the oxidative stress HTL bands proves to be of practical interest.

TL technique is one of the complementary techniques for the study of PS II in normal plants and mutants under growth and developmental stages through scores of environmental conditions. However, application of TL techniques to leaf photosynthesis have been limited due to instrumental constraints and to a lack of understanding of in vivo signals. Several reviews already exist on photosynthesis TL (Sane and Rutherford, 1986; Vass and Inoue, 1992; Misra *et al*., 2001; Tyysjarvi and Vass, 2003). The present review will be focused on TL emission by leaves under different environmental stress conditions depicting the changes in the thylakoid membrane architecture and the molecular organization of the PS II.

THYLAKOID ORGANIZATION AND TL GLOW PEAK

Manganese oxidation states S_2/S_3 were found to be the most luminescent states. The different TL peaks attributed to $S_2Q_A^-$ and $S_2Q_B^-$ recombinations. A damage in the Q_B of PSII during photoinhibition, an increase of the Q-band and the associated C-band (Misra *et al.*, 1998a; Janda *et al*., 1992). The A band (~ +15 °C) is prominent in leaf with damaged oxygen evolving complex. However, frozen samples show Q and A bands, which are reported to be artifacts (Homann, 1999).

CHANGES IN TL GLOW PEAKS UNDER STRESS

TL is a useful tool for the study of photosynthetic electron transfer both at the acceptor and the donor sides of PS II (Misra *et al.,* 2001a, b; Misra and Ramaswamy, 2001). TL peak temperature and intensity is affected by biotic and abiotic stress. Even the developmental factors like etiolation and deetiolation affect the TL properties. Major TL bands are missing in etiolated leaves. Intermittent illumination to greening leaves which do not develop Mn clusture properly, do not show the TL bands (Inoue *et al.,* 1976; Sane *et al.,* 1977). Misra *et al.* (1998b) reported a gradual increase in the Q and B band from base to apex of that wheat leaves greening under continuous illumination, which confirms a developmental gradient across the wheat leaf lamina and a gradual development and organization of the photosynthetic PSII complexes. Leaf aging under continuous light or under continuous darkness resulted in a decrease in Q and B bands (Joshi *et al*., 1993). TL properties in aging leaves clearly depict a decrease in the quinone pool and a block in the electron flow from Q_A to Q_B (Biswal *et al*., 2001).

Biotic stress factors, such as viral infection of the leaves alter appearance of B-band to a higher temperature and the intensity decreases (Rahoutei *et al*., 1999). A new TL peak at 70 °C, due to membrane lipid peroxidation by hypersensitive reactions, appears under these conditions (Stallaert *et al*., 1995).

Photoinhibition of photosynthesis which results in the decrease in quantum efficiency of PSII and degradation of Q_B binding polypeptide affects B-band with least effect on C-band (Misra *et al.*, 1997; 1998a). High temperature induced a decrease in Q- and B-band intensity in leaves (Misra *et al*., 1997, 1998a). Drought stress shifts the redox potential of electron acceptors and so there is shift in the TL glow peaks in the stressed leaves (Ducruet and Vavilin, 1999; Janda *et al.*, 1999). Salt stress affects both the Q and B band in leaves in a dose and duration dependant manner (Biswal *et al.,* 2002; Sahu *et al*., 1999; Misra *et al*., 1998c). The B band is relatively more affected than the Q band. Besides this, there is a back flow of electrons in the salt stressed leaves characterized by the changes in the TL glow peak characters (Zurita *et al*., 2005). Heavy metals copper, nickel, cobalt or zinc affects B band only (Mohanty *et al*., 1989). On the other hand, inert gases like nitrogen, helium, argon or xenon and anoxia reduces the B and C band intensities in leaves (Soltnev *et al*., 1999).

CONCLUSION AND FUTURE PERSPECTIVE

Thermoluminescence in leaves is used for characterizing the charge recombinations in PSII. TL methods are also used as a non-invasive method for the detection of genetic lesions or

genetic modifications in the plants (Debus *et al.*, 1988) which also gave an insight to the origin of different TL bands originating at different temperatures.

It has been used under stress studies extensively to characterize the changes in both the donor side and acceptor side of PS II in stressed leaves. TL studies give insight to PS II structure and function, and also the oxidative state of thylakoid membranes. This tool and technique is also extended to the use in 'sensors' (Zhang *et al*., 2007). As this technique gives an array of information about the electron donors, acceptors and charge accumulation in PS II in chloroplasts of green leaves, no doubt TL studies have an extensive and wide use in photosynthesis and plant biology.

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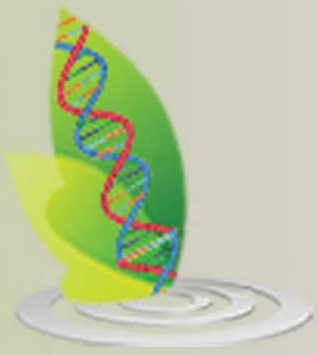

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