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EDITORIAL

Physiologia Plantarum

A tribute to Jean-Marc Ducruet for his contribution to thermoluminescence and photosynthesis research

This special issue is dedicated to Jean-Marc Ducruet whose most relevant contributions to photosynthesis research have been the use of the thermoluminescence (TL) technique and the construction of many TL apparatus for a number of laboratories in different countries. Among the many devices that Jean Marc has built are those of the CEA Saclay in France, that of the University of Seville in Spain, and that of the Center of Agriculture of the Hungarian Academy of Sciences of Martonvásár in Hungary. Jean-Marc Ducruet liked to propagate the TL method, to help especially young scientists, to travel and visit the laboratories of his friends and to construct for them TL devices.

TL in photosynthesis is a technique that is mainly used to study charge recombination reactions in photostystem II (PSII). Although this technique is used by a relatively small number of scientists, it has been shown to have several advantages compared to complex fluorescence decay kinetics since in TL, distinct bands with a characteristic maximum temperature can be easily assigned to the charge pair involved.

Oxygenic photosynthetic organisms emit a long-lived red light during the dark period following an illumination (Amesz & van Gorkom, 1978; Strehler & Arnold, 1951). This light emission, termed as delayed light emission (DLE), is chlorophyll fluorescence originates from PSII, in which the recombination of previously light-separated charge pairs leads, in part, to a radiative process (Amesz & van Gorkom, 1978). Charge pairs photochemically separated stabilizes on PSII by activation energy barriers that limit charge recombination. Charge recombination proceeds following different pathways, one of these leading to the recreation at a low yield of an exciton in the chlorophyll antenna, with a probability to deactivate as fluorescence (Rappaport et al., 2005).

The light-generated charge pairs precursors of DLE can stabilize at low temperature, and the subsequent heating in the dark resulted in DLE, which in this case is generally referred to as TL or thermally stimulated delayed luminescence (Arnold & Sherwood, 1957; Tollin & Calvin, 1957). Decay phases of DLE can be better resolved using TL emission technique (Desai et al., 1983; Miranda & Ducruet, 1995a). This technique consists in recording luminescence emission during the warming of a sample after an irradiation given at a temperature sufficiently low to make negligibly small the recombination rate of the charge pairs under investigation (Ducruet, 2003; Ducruet & Vass, 2009; Inoue, 1996; Sane et al., 2012; Vass & Govindjee, 1996; Vass & Inoue, 1992).

TL technique allows identifying the different types of charge pairs as successive emission bands by a progressive warming. After a sequence of short flashes, a so-called B-band of TL, peaking around 25°C, is observed in both isolated photosynthetic membranes and intact systems. This emission is due to the recombination of $S_2/S_3Q_B^-$ pairs (Demeter et al., 1982; Demeter & Vass, 1984; Inoue & Shibata, 1982; Rutherford et al., 1982; Rutherford et al., 1984). Treatment by a PSII-inhibiting herbicide (diuron, atrazine), which blocks the Q_A to Q_B electron transfer, induces the appearance of a Q-band peaking at about 5°C, due to the $S_2Q_A^-$ recombination (Demeter et al., 1982; Demeter & Vass, 1984). A C-band at about 55°C can also be detected in particular conditions. This TL band is due to $D^+Q_A^-$ recombination (Demeter et al., 1993; Desai et al., 1975; Johnson et al., 1994), D^+ being the oxidized form of tyrosine D on the inactive branch of PSII.

Another luminescence emission is the so-called afterglow (AG) band, which is usually observed in intact photosynthetic materials after far-red (FR > 700 nm) irradiation (Bertsch & Azzi, 1965; García-Calderón et al., 2019; Miranda & Ducruet, 1995b; Roncel et al., 2016; Roncel & Ortega, 2005). This emission was first observed as a delayed burst of luminescence superimposed over the exponential luminescence decay (corresponding to B-band emission in TL) recorded at a constant temperature (Bertsch & Azzi, 1965). When revealed by TL, it corresponds to a sharp band peaking at about 45°C (Miranda & Ducruet, 1995b). AG emission results from a heat-induced back-flow of electrons from reductants present in the stroma to PSII centers initially in the non-recombining state $S_{2/3}Q_B$ (Havaux, 1996; Miranda & Ducruet, 1995b; Sundblad et al., 1988). This charge pair enables to emit AG luminescence as soon as a back-electron transfer from stroma reduces Q_B.

In stressed photosynthetic organisms, strong TL bands can be observed above 60°C without prior illumination. These chemiluminescence high-temperature (HTL) bands are unrelated to charge recombination reactions in PSII with the exception the luminescence emission is in the red, showing that chlorophyll molecules are responsible for it. HTL with a main emission at a maximum at 130°C have been described for algae and leaves submitted previously to oxidative stress. Ducruet published together with Vavilin in 1999 (Ducruet & Vavilin, 1999) that this band correlates well with the content of lipid peroxides. The 130°C HTL band can be used as an indicator of oxidative stress (Havaux & Niyogi, 1999) and has been described by Ducruet as an "ecophysiological indicator" (Ducruet, 2003). A potential application of this slow luminescence component is the imaging of oxidative stress in whole leaves with a highly sensitive CCD camera (Havaux et al., 2006).

All articles of this special issue used TL to study different photosynthetic systems from cyanobateria to higher plants. These articles demonstrate the potential of the TL method for such different scientific questions like characterization of photosynthetic electron transport in mutants and response to special physiological conditions in wild-type plants. The review by *Ortega and Roncel* focusses on the AG-band. Jean-Marc Ducruet demonstrated the role of stromal reductants, chlororespiration and cyclic electron flow for the appearance of the AG-band. The AG-band is a long-lived luminescence emitted from PSII. The occurrence and intensity of this band has been shown to depend on the reduction of the plastoquinone pool in the dark and therefore on the metabolic state of the chloroplast. This band is easiest to see after far-red illumination, exciting preferentially PSI. AG emission corresponds to the fraction of PSII centers in the $S_{2/3}Q_B$ non-radiative state immediately after pre-illumination, in which the arrival of an electron transferred from stroma along produces the $S_{2/3}Q_B^-$ state that emits luminescence. The AG emission recorded by TL technique has been proposed as a simple tool to investigate the chloroplast energetic state and some of its related metabolism processes such as cyclic transport of electrons around PSI and chlororespiration. This review points out not only the basis for the AG emission but also its relevance for the study of certain metabolic pathways like CAM, photorespiration and abiotic and biotic stress responses.

An article demonstrates the existence of this AG-band in cyanobacteria. This band had only been previously detected in algae and plants. *Kodru and collaborators* have characterized for the first time the AG-band in the cyanobacterium *Synechocystis* PCC 6803 by optimizing the temperature for far-red light illumination to the organism. This band is a useful indicator of the presence of cyclic electron flow, which is mediated by the NADH dehydrogenase-like (NDH) complex in higher plants. Although NDH-dependent cyclic electron flow occurs, the AG-band has not been previously found in cyanobacteria. In the present study, the authors have been able to identify a TL component at 40°C, which could be observed when cells were grown under ambient air level CO_2, but was very small, or absent in high CO_2 (3%) grown cells, and in the M55 mutant, which is deficient in the NDH-1 complex. These experimental observations match the characteristics of the AG-band of higher plants. Therefore, the use of the TL technique has allowed the authors to conclude that the newly identified TL component at 40°C in *Synechocystis* PCC 6803 is the cyanobacterial counterpart of the plant AG band, and originates from NDH-1-mediated cyclic electron flow.

TL has proven to be a useful method for testing PSII activity in intact photosynthetic systems. One of the tools used to study the structure–function relationship of PSII has been the construction of algae and cyanobacteria mutants. TL is especially suitable for characterizing PSII function in such mutants, since it can be applied to intact cells without the need for isolation methods. In the paper by *Sugiura and colleagues*, TL has been used to study the PsbA3/R323E site-directed mutant and investigate whether the R323 amino acid of the D1 protein could contribute to regulating a proton exit pathway from the Mn_4CaO_5 and TyrZ group through a proton channel identified from the 3D structure. To test this suggestion, the properties of PSII from this mutant have been compared to those of PsbA3-PSII using EPR, spectroscopy, polarography, TL, and time-resolved UV–visible absorption spectroscopy. TL measurements have revealed that the $S_2Q_A^-$/DCMU and $S_3Q_A^-$/DCMU radiative charge recombination occurred at higher temperatures, which has led to the conclusion, along with other results, that the R323 residue of the D1 protein interacts with TyrZ likely via the H-bond network previously proposed to be a proton channel. This article shows that the TL is a useful tool to characterize mutants in cyanobacteria.

Castell and coworkers investigated the effect of expression of a functional green alga plastocyanin in the diatom *Phaeodactylum tricornutum*. Under iron-deficient conditions, they observed an increased growth and a higher maximum quantum yield of both PSII and PSI, showing the functionality of the heterologous plastocyanin. TL experiments revealed in both, wild type and in the mutant expressing plastocyanin the usual B1- and B2-band ($S_2Q_B^-$ and $S_3Q_B^-$ recombination) at the same temperature. Under Fe-deprivation, the loss in the TL intensity was much larger in the wild type. HTL was used to follow differences in lipid peroxidation in the two strains. In wild type a TL broad band with an emission maximum between 130–140°C was observed indicative for lipid peroxidation products. This band was significantly lower in the plastocyanin-expressing strains. This publication shows that TL is a useful tool to characterize mutants in diatoms in respect to PSII energetics and lipid peroxidation status.

Podmaniczki and coworkers investigated the function of ascorbate on the integrity of the oxygen-evolving complex of PSII (OEC) in *Arabidopsis thaliana* plants treated by prolonged darkness. They used wild type and mutant lines that lack the PsbO1 and PsbR OEC subunits or the key enzyme in the pathway for ascorbate biosynthesis, GDP-L-galactose phosphorylase (vtc2-4 mutant). The TL B-band was used as a tool to follow inactivation of the OEC. The authors found that the inactivation of OEC due to prolonged darkness was attenuated in the ascorbate deficient vtc2-4 mutant, and suggested that ascorbate may actually over-reduce the Mn-cluster in vivo. The severe photosynthetic phenotype of psbO1 knockout mutant was further aggravated upon the prolonged dark treatment. The double psbO1 vtc2 mutant showed a slightly milder photosynthetic phenotype than the single psbO1 mutant did. These results suggested that in the absence of the PsbO1 protein, the Mn-cluster becomes accessible to ascorbate; thereby it can exert a reducing effect resulting in the inactivation of OEC. In conclusion, the authors proposed that upon prolonged darkness, the binding of the extrinsic OEC proteins might be weakened; thereby ascorbate gains access to the Mn-cluster and induces its inactivation by overreduction. They also suggested that PsbO1 and possibly PsbR have a role in vivo in protecting the OEC by hindering the access of ascorbate to Mn-cluster.

The PSII is a sensitive site of the photosynthetic apparatus, which is affected by different environmental factors. This has made TL a powerful tool to study the effects produced in PSII by different stress conditions. *Janda and colleagues* investigated the acclimation response of photosynthetic processes and metabolite concentrations to elevated temperatures in winter and spring varieties of barley, wheat, and oat. Heat acclimation increased the thermotolerance of the

photosynthetic apparatus in all varieties with no significant difference between the winter and the spring varieties. According to their data, heat priming itself does not require general induction of primary metabolites but that the induction of specific routes, for example, the synthesis of galactinol, may contribute the improved heat tolerance in barley and oat leaves. TL measurements did not reveal a significant difference induced by heat acclimation between the cereals used in this study.

Doneva and coworkers investigated the effects of osmotic stress in two wheat varieties with different levels of drought tolerance: the drought-tolerant Katya and drought-sensitive Zora cultivars. They observed that Katya variety exhibited higher constitutive levels of the signaling molecules putrescine and salicylic acid. The tolerance of Katya variety under osmotic stress conditions was characterized by higher photosynthetic ability, stable charge separation in PSII, higher proline accumulation and antioxidant activity. TL technique also revealed significant differences between the two varieties under osmotic stress conditions. In Katya variety, the B-band remained almost unchanged, while a decrease of B-band intensity accompanied by 3°C temperature downshift was found in the drought-sensitive Zora, as well as an increase in AG-band, and a new stress-induced C band appeared. The authors propose that the increase of the AG-band intensity can be explained by a higher capacity for cyclic electron pathways and by assimilatory potential accumulation in chloroplasts in conditions of lack of CO_2 due to stomatal limitations. The authors also characterized the effect of pre-treatment with the polyamine putrescine on osmotic stress response in the two wheat varieties. They observed a significant increase in photosynthetic activity, stomatal conductance, and transpiration, which was more pronounced in the tolerant Katya variety. Putrescine pre-treatment also increased the activity of the antioxidant enzymes catalase and ascorbate peroxidase to a higher level in Katya variety. However, a significant increase in putrescines level was only observed in the leaves of Zora variety. Taking into account these results, the authors finally discussed the possibility to use putrescine pre-treatment as a promising and beneficial agricultural practice to increase stress resistance of crops.

In the article by *Leverne and Krieger-Liszkay*, the TL technique has been used to characterize the effect of drought on the intensity and maximum temperature of the AG- and B- bands. Under moderate drought conditions, an increase of the TL AG-band and a downshift of the maximum temperatures of both, the B-band and the AG-band, were observed when leaves were illuminated under conditions that maintained the proton gradient. When leaves were frozen prior to the TL measurements, the maximum temperature of the B-band was upshifted in drought-stressed leaves, indicating a stabilization of the $Q_B/Q_B^{\bullet-}$ redox couple in accordance with the slower fluorescence decay kinetics. These results have allowed the authors to propose that during drought stress, photorespiration exerts a feedback control on PSII. It is proposed that this control is carried out by the binding of a photorespiratory metabolite glycolate at the non-heme iron at the acceptor side of PSII, affecting not only the midpoint potential of the $Q_A/Q_A^{\bullet-}$ couple but also that of the $Q_B/Q_B^{\bullet-}$ couple.

The work by *Rac and coworkers* promotes the understanding of the mechanism of lipid peroxidation in leaves, a primary event associated with oxidative stress in plants. This study characterized the reactive carbonyl species (RCS) secondarily generated by lipid peroxidation in *Arabidopsis* plants exposed to photo-oxidative stress conditions. The authors also investigated the effects of exogenous applications of RCS by autoluminescence-imaging techniques. They used three different genotypes: the wild type, the *scl14* knockout mutant and *scl14* overexpressing transgenic line (OE:SCL14). Scarecrow-like 14 (SCL14) transcription regulator is part of TGAII/SCL14 complex that governs the expression of several detoxifying enzymes in cells, as alkenal reductase (AER), which target RCS. The authors identified that some of RCS, especially 4-hydroxynonenal (HNE), and to a lesser extent 4-hydroxyhexenal (HHE), as highly reactive compounds that are harmful to leaves and can trigger *AER* gene expression, contrary to other RCS (pentenal, hexenal) and to isoprostanoids. They showed that exogenously applied HNE was similarly damaging to the *scl14* mutant, its wild-type parent and the OE:SCL14 transgenic line. However, strongly boosting the SCL14 detoxification pathway and AER expression by a pre-treatment of OE:SCL14 with the signaling apocarotenoid β-cyclocitral (βCC) canceled the damaging effects of HNE. β-CC is produced under stress conditions by oxidation of β-carotene by singlet oxygen in the PSII reaction centers, and can trigger the detoxification mechanism controlled by TGAII/SCL14 complex. These results indicate that the cellular detoxification pathway induced by the low-toxicity β-cyclocitral targets highly toxic compounds produced during lipid peroxidation. This study has also illustrated the usefulness of autoluminescence imaging to monitor RCS induced oxidative degradation of leaf tissues even when visual symptoms and leaf necroses are hardly visible.

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