PHOTOSYNTHETICA 40 (1): 153-156, 2002

BRIEF COMMUNICATION

Whole leaf thermoluminescence emission in *Pisum sativum* L.: influence of leaflet age

M. ROMAN

Lasers Department, INFLPR, P.O. Box MG-36, Bucharest-Magurele, Romania

Abstract

The thermoluminescence signals from leaflets of the same pea plant varied gradually according to their developmental stage. The AG emission, due to a back flow of electrons towards photosystem 2 (PS2) along a cyclic/chlororespiratory pathway, was stronger in mature leaves than in the growing ones. These age-related variations could be explained by a higher capacity of cyclic electron flow in mature leaves.

Additional key words: "afterglow" emission; pea; photosystem 2.

Chlorophyll photosynthetic luminescence originates from photosystem (PS) 2 and results from the recombination of charge pairs separated by a prior irradiation. Luminescence decay phases can be better resolved as thermoluminescence (TL), which consists in the recording of luminescence emission during the warming of the sample following an irradiation done at a relatively low temperature (for review see Vass and Inoue 1992). Thermoluminescence reacts on biotic and abiotic stresses (for review, see Misra *et al.* 2001). After a short sequence of flashes, a so-called B-band of TL is classically observed, peaking at about 35 °C in both isolated thylakoids and leaves. It is due to a $Q_B^-S_2/S_3$ recombination (Q_B^- – reduced secondary acceptor; S_2/S_3 – oxygen-evolving complex states, having 2 or 3 positive charges stored on manganese cluster; Demeter and Vass 1984, Rutherford *et al.* 1984). Another luminescence emission, usually obtained after a far-red (FR) irradiation (>700 nm) of an intact photosynthetic material (leaves, algae, intact chloroplasts), is the "afterglow" (AG) emission. Although FR radiation excites mainly PS1, its weak absorption by the PS2 antenna is sufficient to randomly generate S_2/S_3 luminescence-emitting states. This emission was first observed as a delayed burst of luminescence superimposed over the exponential luminescence decay (corresponding to the B type emission in TL), recorded at a constant temperature (Bertsch and Azzi 1965). It corresponds to a sharp band peaking at about 45 °C (Desai *et al.* 1983, Nakamoto *et al.* 1988, Miranda and Ducruet 1995a), when revealed by TL. Similarly as the B band, the AG emission results from a $Q_B^-S_{2/3}$ charge recombination within PS2 centres (Hideg *et al.* 1991) excited by the weakly absorbed FR radiation; the difference between B and AG emissions is apparently due to the Q_B reduction pathway (Sundblad *et al.* 1989). The AG emission originates from those PS2 centres in which the oxidised Q_B is progressively reduced during TL warming, by a reverse electron flow from stromal reductants toward the plastoquinone pool and Q_B. Such flow occurs in the presence of a transthylakoidal proton gradient (Björn 1971, Sundblad *et al.* 1989).

It has been shown by photoacoustic spectroscopy that short heat treatments in the same range of temperature as that of AG emission (about 45 °C) trigger a PS1-driven electron flow from stromal reductants towards the acceptor side of PS2 (Havaux 1996). This flow can proceed in the dark along the same pathway, as a part of the chlororespiratory process, which occurs in higher plants (Garab *et al.* 1989). This heat-induced reduction of Q_B gives rise to the AG emission through recombination with the S_2/S_3 states.

The AG emission can be used to define physiological indicators of the energetic potential [NADPH+ATP] and

---

Received 25 October 2001, *accepted* 28 February 2002.

Fax: 004014574467, e-mail: mirunaroman@yahoo.com

Abbreviations: AG – "afterglow" or delayed luminescence rise and the corresponding TL band; B band – TL band due to S_2/S_3 Q_B^- recombination; FQR – ferredoxin plastoquinone reductase; FR – far red; NDH – NAD(P)H-deshydrogenase (plastoquinone reductase); PS – photosystem; TL – thermoluminescence; T_m – temperature corresponding to the maximum of a TL band.

Acknowledgement: I thank Dr. J.-M. Ducruet for helpful discussions.

of the induction of cyclic electron flow *in vivo* (Mellvig and Tillberg 1986, Sundblad 1988, Krieger *et al.* 1998, Roman and Ducruet 2000). In the present paper we show that this emission depends on the age of pea leaflets, similarly as fluorescence intensity (Šesták and Šiffel 1997, Šesták 1999), in a way that can be related to the leaflet physiological sink/source role (Stitt *et al.* 1988).

Pea (*Pisum sativum* L. cv. Merveille de Kelvedon) plants were grown up to a 9 leaves-stage (2 weeks after emerging) on *Perlite*, fertilised with a *Hydrokani*® *H2* nutritive solution, in a *Conviron E7* chamber (Winnipeg, Canada), with a 16/8 h 18/14 °C (day/night) cycle. Fluorescent tubes and halogen lamps provided irradiation, with a photon flux density of 230 µmol m^{-2} s^{-1} at the leaf level. Measurements were done on disks of leaflets at different developmental ages of the same dark-adapted plant. Freshly excised 25 mm diameter leaflet disks were softly pressed against the thermostated Peltier plate in the TL sample holder, with their adaxial face oriented towards the photomultiplier tube.

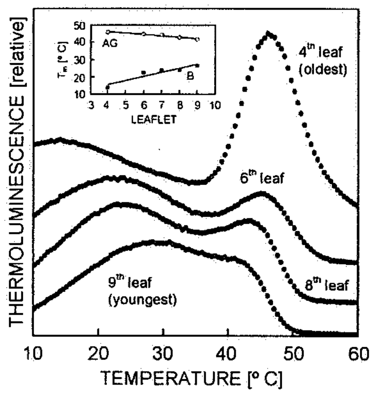

Fig. 1. Thermoluminescence (TL) signals obtained after a 30-s FR irradiation at 0 °C on dark-adapted pea leaflets of different ages from the same plant. Leaves counting started from root level, with the oldest leaflet. *Insert*: T_mB and T_mAG (temperature corresponding to the maximum of the B or AG band, respectively) dependence on the leaflet age. (For clarity, the TL signals of the 5th and the 7th leaflets were omitted in this figure.)

For pre-irradiated samples, a 3-min actinic irradiation (600 µmol m^{-2} s^{-1} at 20 °C, "white light") of leaf disks was followed by 10-min dark-adaptation at 20 °C outside the TL holder. Pre-irradiated or dark-adapted samples were kept in the dark on the Peltier plate for 2 min, at 20 °C. Then they were cooled to 0 °C for 60 s, with an FR-irradiation during the last 30 s (*Walz 102-FR*, set at 10), immediately before starting the TL recording (from 0 to 70 °C, with a 0.5 °C per s heating rate). The TL-measuring device was a laboratory-made set-up previously described (Miranda and Ducruet 1995a, Ducruet *et al.* 1998). Basically, the sample temperature was determined by a thermoelectric *Marlow* (Dallas, USA) *DT-1089* plate (maximum range: from –15 to +100 °C) and luminescence was detected by a compact *Hamamatsu* (Tokyo, Japan) *H5701-50* red-extended photomultiplier.

The TL signal was decomposed into elementary bands to evaluate the maximal temperatures T_m of the B and AG bands using a numerical simulation (Ducruet and Miranda 1992).

Significant differences in the intensities and positions of AG and B bands were observed between leaflets of different ages from the same plant. Actually, the AG band intensity was higher for a mature leaflet as compared to a younger one of the same dark-adapted plant (Fig. 1). Moreover, the maximum temperature T_m(B) of the B-band was increasingly downshifted from growing to mature leaflets (Fig. 1, *insert*). A 3-min actinic ("white light") pre-irradiation stimulated the AG emission and apparently decreased the B-band in the growing leaflets (Fig. 2*A*), whilst negligible changes were observed in the mature ones (Fig. 2*B*). Pre-irradiation also enhanced the FR-induced downshift of the B-band in growing leaflets, which can be explained by a decreased stabilisation of positive charges in S_2/S_3 states exposed to an acidic lumen (Joliot and Joliot 1980, Miranda and Ducruet 1995b). This indicates a stronger thylakoid lumen acidification after FR pre-irradiation as compared to dark-adapted growing leaflets. Prior exposure to an actinic radiation restored an acidification of the thylakoid lumen by FR light as well as it increased the AG-band intensity in growing leaflets. This relation between the intensity of the AG-band and the downshift of the B-band is consistent with the requirement of a trans-thylakoidal proton gradient necessary to the reverse electron flow, which produces the AG emission (Sundblad *et al.* 1989). We

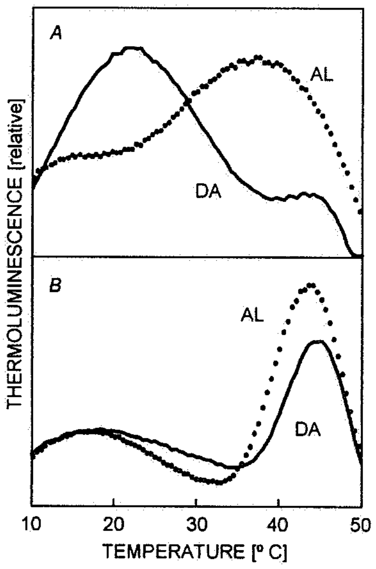

Fig. 2. Thermoluminescence signals obtained after a 30 s far-red irradiation at 0 °C on dark-adapted leaflets (DA) and on leaflets, which were pre-irradiated for 3 min by "white actinic light" (AL). Growing leaflet (*A*) and mature leaflet (*B*).

performed 5 replicates of the experiments to confirm that the results were qualitatively reproducible.

Taking into account the above mentioned mechanism of the AG emission and our observations of the characteristics of the TL signals for leaflets in different developmental stages, we suggest that the AG-band intensity might depend on the [NADPH+ATP] energetic potential (Krieger *et al.* 1998), smaller in dark-adapted growing leaflets (sink) and higher in mature leaflets (source). An actinic pre-irradiation significantly increases the [NADPH+ATP] energetic potential in growing leaflets, whilst this effect is less pronounced in mature leaflets, in which this potential is already large in dark-adapted conditions. The AG emission is dependent on the cyclic electron flow. However, it is unaffected in tobacco mutants lacking NDH activity (A. Liszkay-Krieger, personal communication; Cournac and Ducruet, unpublished), whereas it is suppressed by 5 µM antimycin A, a low concentration of which inhibits the ferredoxin plastoquinone reductase (FQR) activity but not the NDH pathway (Scheller 1996, Endo *et al.* 1998). In *ndh*B-mutants of tobacco, the cyclic electron flow measured by the re-reduction of P700 is fully suppressed in growing leaves, but partly remains in mature leaves (Shikanai *et al.* 1998). Hence, this residual cyclic electron flow and the AG emission are both independent on NDH activity and probably reflect another cyclic pathway, which can be tentatively identified as the FQR pathway.

References

Bertsch, W.F., Azzi, J.R.: A relative maximum in the decay of long-term delayed light emission from the photosynthetic apparatus. – Biochim. biophys. Acta **94**: 15-26, 1965.

Björn, L.O.: Far-red induced, long-lived afterglow from photosynthetic cells. Size of afterglow unit and paths of energy accumulation and dissipation. – Photochem. Photobiol. **13**: 5-20, 1971.

Demeter, S., Vass, I.: Charge accumulation and recombination in photosystem II studied by thermoluminescence. I. Participation of the primary acceptor Q and secondary acceptor B in the generation of thermoluminescence of chloroplasts. – Biochim. biophys. Acta **764**: 24-32, 1984.

Desai, T.S., Rane, S.S., Tatake, V.G., Sane, P.V.: Identification of far-red-induced relative increase in the decay of delayed light emission from photosynthetic membranes with thermoluminescence peak V appearing at 321 K. – Biochim. biophys. Acta **724**: 485-489, 1983.

Ducruet, J.-M., Miranda, T.: Graphical and numerical analysis of thermoluminescence and fluorescence F_0 emission in photosynthetic material. – Photosynth. Res. **33**: 15-27, 1992.

Ducruet, J.-M., Toulouse, A., Roman, M.: Thermoluminescence of plant leaves: Instrumental and experimental aspects. – In: Garab, G. (ed.): Photosynthesis: Mechanisms and Effects. Vol. V. Pp. 4353-4356. Kluwer Academic Press, Dordrecht – Boston – London 1998.

Endo, T., Shikanai, T., Sato, T., Asada, K.: NAD(P)H dehydrogenase-dependent, antimycin A-sensitive electron donation to plastoquinone in tobacco chloroplasts. – Plant Cell Physiol. **39**: 1226-1231, 1998.

Garab, G., Lajkó, F., Mustárdy, L., Márton, L.: Respiratory control over photosynthetic electron transport in chloroplasts of higher-plant cells: evidence for chlororespiration. – Planta **179**: 349-358, 1989.

Havaux, M.: Short-term responses of Photosystem I to heat stress. Induction of a PS II-independent electron transport through PS I fed by stromal components. – Photosynth. Res. **47**: 85-97, 1996.

Hideg, É., Kobayashi, M., Inaba, H.: The far red induced slow component of delayed light from chloroplasts is emitted from Photosystem II. Evidence from emission spectroscopy. – Photosynth. Res. **29**: 107-112, 1991.

Joliot, P., Joliot, A.: Dependence of delayed luminescence upon adenosine triphosphatase activity in *Chlorella*. – Plant Physiol. **65**: 691-696, 1980.

Krieger, A., Bolte, S., Dietz, K.-J., Ducruet, J.M.: Thermoluminescence studies on the facultative crassulacean-acid-metabolism plant *Mesembryanthemum crystallinum* L. – Planta **205**: 587-594, 1998.

Mellvig, S., Tillberg, J.-E.: Transient peaks in the delayed luminescence from *Scenedesmus obtusiusculus* induced by phosphorus starvation and carbon dioxide deficiency. – Physiol. Plant. **68**: 180-188, 1986.

Miranda, T., Ducruet, J.-M.: Characterization of the chlorophyll thermoluminescence afterglow in dark-adapted or far-red-illuminated plant leaves. – Plant Physiol. Biochem. **33**: 689-699, 1995a.

Miranda, T., Ducruet, J.-M.: Effects of dark- and light-induced proton gradients in thylakoids on the Q and B thermoluminescence bands. – Photosynth. Res. **43**: 251- 262, 1995b.

Misra, A.N., Dilnawaz, F., Misra, M., Biswal, A.K.: Thermoluminescence in chloroplasts as an indicator of alterations in photosystem 2 reaction centre by biotic and abiotic stresses. – Photosynthetica **39**: 1-9, 2001.

Nakamoto, I., Sundblad, L.G., Gardenström, P., Sundborn, E.: Far-red stimulated long-lived luminescence from barley protoplasts. – Plant Sci. **55**: 1-7, 1988.

Roman, M., Ducruet, J.M.: Evidence from leaf thermoluminescence for a decrease of the [NADPH + ATP] energetic potential in cold-sensitive *Pisum sativum* L. varieties upon hardening at 5 °C. – J. Plant Physiol. **157**: 177-181, 2000.

Rutherford, A.W., Renger, G., Koike, H., Inoue, Y.: Thermoluminescence as a probe of Photosystem II. The redox and protonation states of the secondary acceptor quinone and O_2-evolving enzyme. – Biochim. biophys. Acta **767**: 548-556, 1984.

Scheller, H.V.: *In vitro* cyclic electron transport in barley thylakoids follows two independent pathways. – Plant Physiol. **110**: 187-194, 1996.

Šesták, Z.: Chlorophyll fluorescence kinetic depends on age of leaves and plants. – In: Argyroudi-Akoyunoglou, J.H., Senger, H. (ed.): The Chloroplast: From Molecular Biology to Biotechnology. Pp. 291-296. Kluwer Academic Publ., Dordrecht – Boston – London 1999.

Šesták, Z., Šiffel, P.: Leaf age-related differences in chlorophyll fluorescence. – Photosynthetica **33**: 347-369, 1997.

Shikanai, T., Endo, T., Hashimoto, T., Yamada, Y., Asada, K., Yokota, A.: Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. – Proc.

nat. Acad. Sci. USA **95**: 9705-9709, 1998.

Stitt, M., Wilke, I., Feil, R., Heldt, H.W.: Coarse control of sucrose-phosphate synthase in leaves: Alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. – Planta **174**: 217-230, 1988.

Sundblad, L.-G.: Dark reduction of Q_A in intact barley leaves as an effect of lowered CO_2 concentration monitored by chlorophyll *a* luminescence and chlorophyll *a* F_0 dark fluorescence. – Biochim. biophys. Acta **936**: 429-434, 1988.

Sundblad, L.-G., Schröder, W.P., Åkerlund, H.-E.: S-state distribution and redox state of Q_A in barley in relation to luminescence decay kinetics. – Biochim. biophys. Acta **973**: 47-52, 1989.

Vass, I., Inoue, Y.: Thermoluminescence in the study of photosystem II. – In: Barber, J. (ed.): The Photosystems: Structure, Function and Molecular Biology. Pp. 259-294. Elsevier Science Publishers, Amsterdam – London – New York – Tokyo 1992.