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Thermoluminescence investigation of low temperature stress in maize

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Abstract

The thermoluminescence (TL) emission of photosynthesising materials originates from the recombination of charge pairs created by a previous excitation. Using a recently described TL set-up the effect of chilling stress on TL bands occurring at positive temperatures (AG, B, and HTL) was investigated in intact leaves. The far-red irradiation of leaves at low, but non-freezing temperatures induced a TL band peaking at around 40-45 °C (AG band), together with a B band peaking between 20 and 35 °C. Low temperature stress first caused a downshift and a temporary increase in the AG band after 4 h at 0 °C in the light, then a decrease in the AG and B TL bands after 1 d at 0 °C in the light. This decrease was less pronounced in cold-tolerant genotypes and in those grown at acclimating temperatures. Furthermore, an additional band appeared above 80 °C after severe cold stress. This band indicates the presence of lipid peroxides. Thus TL is a useful technique for studying the effects of low temperature stress.

Additional key words: afterglow; chilling; peroxide; *Zea mays*.

Introduction

The thermoluminescence (TL) emission of photosynthesising materials (leaf, algae cells, chloroplasts, thylakoids, or PS2 particle suspension) originates from the charge recombination between the positively charged donors and negatively charged acceptors of PS2. Charge recombination between the S_2/S_3 states of the water splitting system and Q_B^- gives the B TL band peaking at around 30-35 °C. The addition of diuron (electron transport inhibitor between the Q_A and Q_B quinone acceptors) leads, due to an $S_2Q_A^-$ charge recombination, to the Q TL-band peaking at 5-12 °C (Rutherford *et al.* 1982, Demeter and Vass 1984). For reviews on TL see Inoue (1996) and Vass and Govindjee (1996).

Irradiation of a long-term dark-adapted leaf or intact chloroplasts with far-red radiation induces an afterglow emission (Bertsch and Azzi 1965) which can be optimally resolved as an AG (afterglow) TL band (Miranda and Ducruet 1995a). AG emission is generally weaker or absent after "white light" excitation, although it has occasionally been observed following flash excitation, *e.g.*, in pea or cucumber leaves (Miranda and Ducruet 1995a). However, when algae adapted to a high CO_2 concentration were transferred to a low-CO_2 medium, a "white light"-induced AG peak was observed, reflecting a rise in the energetic potential needed to drive active CO_2 uptake (Mellvig and Tillberg 1986).

The AG band reflects a more complex phenomenon than the B and Q TL bands, which are specific for charge recombination within PS2. Not only PS2, but also part of the cyclic electron pathway and transthylakoid pH gradient are involved in the AG emission, which corresponds to a back electron transfer towards PS2 centres initially in the S_2/S_3 Q_B state (Sundblad *et al.* 1988). Phosphorus and carbon dioxide deficiencies also affect the transient peaks in delayed luminescence induced by far-red radiation, as was shown in *Scenedesmus obtusiusculus* cells (Mellvig and Tillberg 1986). The AG band may reflect the [ATP+NADPH] concentration in the chloroplast, as shown in *Mesembryanthemum crystallinum*, a facultative crassulacean-acid-metabolism plant (Krieger *et al.* 1998).

Low temperature is one of the most important limiting factors in the wider spread of several crop plants (Stamp 1984, Kocsy *et al.* 1996, Marton and Szundy 1997). The far-red induced afterglow emission is very sensitive to temperature stresses. The AG band is a tentative indicator of different types of stress, such as heat, freezing, and drought (Janda *et al.* 1999). The aim of the present study was to investigate the effect of chilling stress on the AG TL band in young maize plants.

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Abbreviations: AG – afterglow; PS – photosystem; TL – thermoluminescence.
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Materials and methods

Plants: Maize plants (*Zea mays* L., hybrid Norma; inbred lines Mo17 and CM 7) were grown for 2-3 weeks in a mixture of loamy soil, *Vegasca* (humus-containing additive, manufactured by *Florasca*), and sand (3 : 1 : 1, v:v:v) at 22/20 °C in a *Conviron PGV-36* plant growth chamber or at 15/13 °C in a *Conviron PGR-15* chamber with 16/8-h light/dark periodicity in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences. The photosynthetic photon flux density (PPFD) at leaf level was 250 μmol m^{-2} s^{-1}, provided by metal halide lamps (*Tungsram*, Budapest, Hungary), and the relative humidity was 75 %. The cold treatment was carried out when the plants were in the 4-leaf stage, in a *PGV-36* chamber at 0 °C, also at 250 μmol m^{-2} s^{-1} PPFD, using the same type of lamps. The temperature was maintained with an accuracy of ±0.2 °C, so there was no danger of temporary freezing. The measurements were carried out on the youngest fully developed leaves.

Thermoluminescence measurements were performed using the laboratory-made apparatus and software described earlier (Miranda and Ducruet 1995a, Ducruet *et al.* 1997). Luminescence was detected by a *Hamamatsu H5701-50* photomultiplier linked to an amplifier. A 4×4 cm Peltier element (*Marlow Instruments*, Dallas, Texas, USA) was used for temperature control. The samples (upper surface of the leaf segments without the central vein) were cooled within a few seconds and irradiated *via* a fiber optic either by far-red radiation provided by a *PAM 102-FR* light source (*Walz*, Effeltrich, Germany) for 30 s (setting: 11) or by a single turnover flash lamp (*XST 103*; *Walz*, Effeltrich, Germany) at 1 °C. The leaf sample was gently pressed against the plate by a rubber ring and a *Pyrex* window, with the addition of 100 mm^3 water for better thermal conduction. The rate of heating during measurement was 0.5 °C s^{-1}. The TL measurements were repeated several times and representative curves are presented in the figures.

Electrolyte leakage measurements were carried out as described by Szalai *et al.* (1996) based on electric conductivity measurements. Two leaf disks (7 mm in diameter) were placed in vials containing 2 cm^3 ultrapure water (*Milli-Q 50*, *Millipore*, Bedford, MA, USA) and were gently shaken for 1 h in the dark. Conductivity was measured using an Automatic Seed Analyzer (*ASA610, Agro Sciences*, Ann Arbor, MI, USA) at room temperature. Electrolyte leakage values are given as a percentage of the fully damaged samples, which were determined after incubation of the leaf disks for 1 d at –80 °C. The results are means of 10 measurements. They were statistically evaluated using the *t*-test method.

Chlorophyll content was determined spectrophotometrically in 100 % acetone extracts according to Lichtenthaler (1987).

Results and discussion

The far-red irradiation of leaves at low but non-freezing temperatures induces a TL band peaking at around 40-45 °C (afterglow or AG-band), together with a B-band peaking below 30 °C (Miranda and Ducruet 1995a). A short-term low temperature treatment (4 h at 0 °C in the light) caused a slight increase and a downshift towards lower temperatures in the AG band in maize plants grown at 22/20 °C (Fig. 1*A*). The B band induced by 1 single turnover flash did not show this increase, although a slight downshift of T_{max} could be observed (Fig. 1*B*). Severe chilling stress caused a dramatic decrease in the AG band. After 1 d of cold treatment carried out at 0 °C in the light, both the B and AG bands disappeared and a band peaking above 80 °C appeared in young maize (hybrid Norma) plants grown at 22/20 °C. This band was especially strong in leaf segments which suffered water loss, a typical symptom of severe cold stress (Miedema 1982), and appeared to have died. TL bands arising above 60 °C are independent of previous irradiation and correlate with the amount of lipid peroxides in the plant sample (Venediktov *et al.* 1989, Hideg and Vass 1993, Vavilin and Ducruet 1998). The appearance of the high temperature TL bands (HTL) showed good correlation with the accumulation of thiobarbituric acid reactive substances (Vavilin *et al.* 1998). Elicitor treatment (Stallaert *et al.* 1995) or virus infection (Rahoutei *et al.* 1999) may also lead to the appearance of HTL, as was shown for example in tobacco species. Moisture in the sample may suppress certain HTL bands (Ducruet and Vavilin 1999). Besides the fact that these leaf segments are expected to contain the highest amount of peroxides, this may also explain why this band was so high in leaves that dried out due to the severe low temperature stress.

The low temperature stress-induced loss of the AG band is affected by several factors. For example, in plants which were grown at relatively low (15/13 °C), acclimating temperature (Janda *et al.* 1998), the AG band was downshifted towards low temperatures, and the decrease occurring after 1 d of chilling stress was less pronounced than in those grown at 22/20 °C (Fig. 1*C*). The T_{max} value of the AG TL band is usually at lower temperature in hardened cold-tolerant genotypes than in the sensitive ones (Ducruet *et al.* 1997).

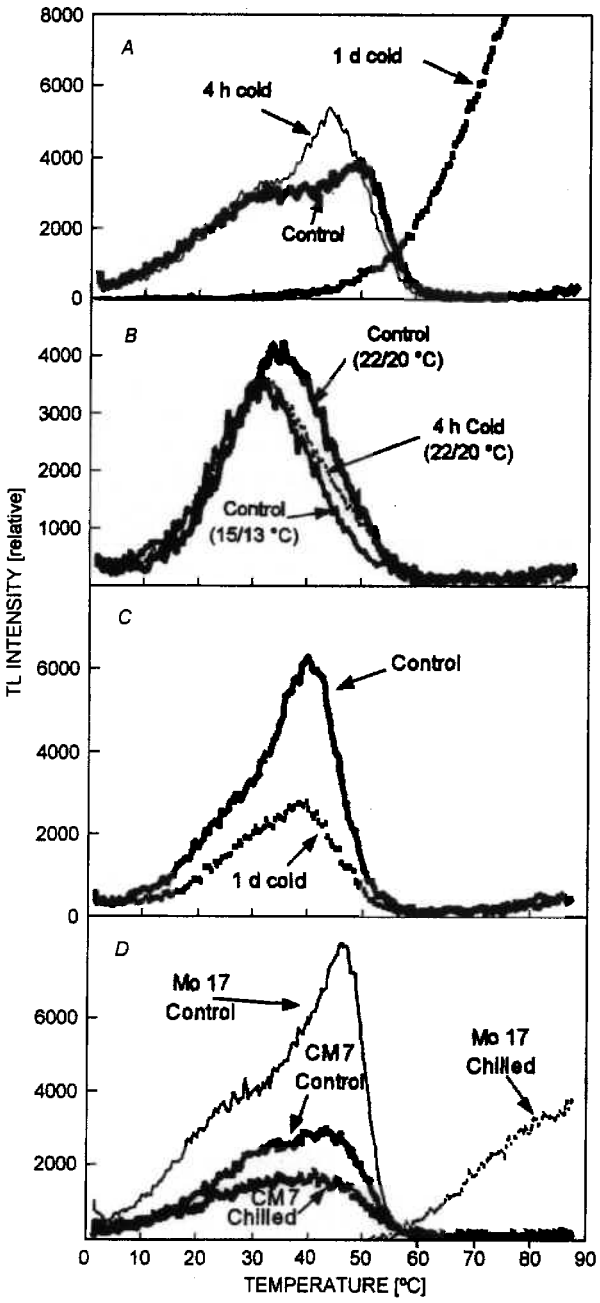

Fig. 1. (*A*) Changes in the TL curve induced by 30 s far-red radiation after 4 h or 1 d low temperature stress (0 °C at 250 μmol m^{-2} s^{-1} PPFD) in plants grown at 22/20 °C. (*B*) Effect of 4 h low temperature (0 °C at 250 μmol m^{-2} s^{-1} PPFD) or low growth temperature (15/13 °C) on the B TL band induced by 1 flash. Temperature values in parentheses indicate the growth temperatures (day/night, with 16 h photoperiod). (*C*) Changes in the TL curve induced by 30 s far-red radiation after 1 d low temperature stress (0 °C at 250 μmol m^{-2} s^{-1} PPFD) in plants grown at acclimating temperatures of 15/13 °C. (*D*) Effect of 1 d low temperature (0 °C at 250 μmol m^{-2} s^{-1} PPFD) on the TL curve induced by 30 s FR radiation in Mo 17 (cold-sensitive) and CM 7 (cold-tolerant) inbred maize. – In *A* to *C* maize plants of hybrid Norma. Samples were always taken from the middle part of the leaves.

Low growth temperature caused a slight downshift in the T_{max} of the B band induced by 1 flash. Downshifts of the B TL band have already been reported both in higher plants and in green algae. In the green alga *Chlamydomonas reinhardtii* the downshift in the B band from 30 to 15-17 °C after photoinhibitory treatment may be a consequence of a conformational change in the D1 protein of PS2 (Ohad *et al.* 1988). In another green alga, *Chlamydobotrys stellata*, and in pea leaves the photoinhibition-induced downshift was attributed to the different extent of reduction in the Q and B bands (Janda *et al.* 1992). Not only photoinhibition but also acidification in the lumen of the thylakoid membrane may lead to a downshift of the B TL band (Miranda and Ducruet 1995b).

Table 1. Means (±SD) of the electrolyte leakage values given as a percentage of totally damaged samples (1 d at –80 °C) in control (grown at 22/20 °C) and low temperature stressed (2 d at 0 °C) cold-tolerant CM 7 and cold-sensitive Mo 17 maize inbred lines. Samples were taken from the middle part of the youngest fully developed leaves. **, ***: significant at the 0.01 and 0.001 levels, respectively, compared to control, not chilled plants ($n = 10$).

Genotype	Control	Cold treated
CM 7	22.3 ± 4.6	31.6 ± 7.7**
Mo 17	23.1 ± 5.9	65.8 ± 16.1***

The intensity of the AG band indicates the NADPH+ATP potential (Krieger *et al.* 1998), which is decreased by low temperature stress, as shown in pea plants (Roman and Ducruet 2000). Besides the photoinhibition of PS2, this decrease may also contribute to the loss of the AG band after chilling in maize. Particularly in

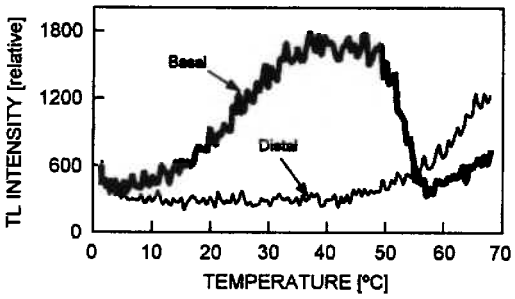

Fig. 2. TL curves induced by 30 s FR radiation from the basal (sampled from the middle of the third closest to the base of the leaf) and distal part (sampled from the middle of the third closest to the tip of the leaf) of leaves of young maize (hybrid Norma) plants after 1 d chilling (0 °C at 250 μmol m^{-2} s^{-1} PPFD).

chilling sensitive plants, not only PS2, but also PS1 may be photoinhibited under low temperature stress (Sonoike 1996, Barth and Krause 1999). Since the cyclic electron

flow around PS1 may be involved in the AG band, the low temperature-induced inhibition of PS1 may also decrease the AG band.

To investigate how changes in the AG band reflect the chilling tolerance of maize plants, two inbred lines, CM 7 (cold-tolerant) and Mo 17 (cold-sensitive) were compared. Electrolyte leakage measurements reflected the difference between the two lines: membrane destruction was much more pronounced in the sensitive Mo 17 line than in the tolerant CM 7 line after cold treatment (Table 1). Measurement of the far-red-induced TL curve showed a similar result: although there was a significant decline in both the B and AG bands even in the cold-tolerant CM 7 line after a 1-d cold treatment at 0 °C, this chilling stress totally suppressed these bands in Mo 17, and a considerable HTL band was detected (Fig. 1*D*). The chlorophyll content was usually 1.3 times higher in Mo 17 line than in CM 7 (values not shown). This higher chlorophyll content may also contribute to the higher TL signal detected from Mo 17.

However, a significant difference can be observed within the parts of the individual plants: for example, the parts of the leaves closer to the base were less sensitive than the distal parts (Fig. 2), which must be taken into account when the aim is to compare different kinds of plants.

In conclusion, the far-red induced TL curve showed complex behaviour during chilling stress. Mild stress may cause a transient increase in the AG band together with a downshift in its T_{max} value. Severe stress progressively decreases both the B and AG bands, which may lead to their complete loss (in contrast to short-term freezing in the dark, after which a substantial part of the B band still remains) and to the appearance of the HTL band(s) indicating the presence of lipid peroxides. Changes in the AG curve depend on the chilling tolerance of the plant.

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