The Thermoluminescence 'Afterglow' Band as a Sensitive Indicator of Abiotic Stresses in Plants

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Single turn-over xenon flashes induce a thermoluminescence (TL) B-band centred near 35 °C. The far-red illumination of leaves at non-freezing temperatures induces a band peaking at around 45 °C (afterglow or AG-band), together with a downshifted B-band peaking between 15 °C and 28 °C. In control, unfrozen wheat plants, the T_{max} of the B-band induced after 30 s far-red light at 0 °C was approx. 15–18 °C. In maize plants grown under the same conditions, this far-red-induced downshift was not so strong, since the B-band peaked at 28–30 °C. Both a decline in the AG-band and a reversal of the downshift of the B-band were observed after short-term freezing in several plant species. There was usually a sudden drop in the AG-band below a critical freezing temperature. However, while in wheat plants a weak TL emission could be seen between 40–50 °C in frozen samples, in cold-sensitive maize plants this was completely suppressed and only the B-band could be detected. In cold-hardened wheat plants the temperature at which the AG-band was suppressed was lower than in non-hardened plants. Drought and short-term heat stress also affect the AG-band. As the AG-band was found to be more sensitive to several types of stresses than the B-band, it can be used as a sensitive stress indicator. However, the behaviour of the AG-band depends on several factors (for example the age of the leaf, etc.), which must be controlled if different species or varieties are to be compared.

Introduction

The brief illumination of a dark-adapted photosynthesizing material (leaf, algal cells, chloroplast, thylakoid or PS II particle suspension) with white light induces a charge recombination between the S_2/S_3 states and Q_B^-, generating a 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 to an $S_2Q_A^-$ charge recombination corresponding to the Q TL-band peaking at 5–12 °C (Rutherford *et al.,* 1982; Demeter and Vass, 1984). For reviews of thermoluminescence, see Inoue (1996) and Vass and Govindjee (1996).

A delayed luminescence rise, or afterglow (AG) induced by far-red (FR) illumination was first reported by Bertsch and Azzi (1965). Earlier studies were performed at constant temperature. However, by slowly heating a dark-adapted plant sample, the AG emission can be resolved as a well-defined thermoluminescence (TL) band peaking at around 45 °C (Miranda and Ducruet, 1995a; Ducruet *et al.,* 1997).

The occurrence of an AG-band reflects a more complex phenomenon than the B and Q TL bands, which represent the back-reaction of a PS-II charge separation. This afterglow emission can be observed only in intact systems, such as leaves (Björn, 1971; Sundblad, 1988), protoplast (Nakamoto *et al.*, 1988) or intact chloroplasts (Hideg *et al.*, 1991), but not in thylakoid or PS II particle suspensions. PS II inhibitors such as diuron not only convert the B-band into a Q-band, but also suppress the AG-band induced by FR light. Furthermore, AG can be inhibited by uncouplers or by antimycin, which inhibits the PS I cyclic electron transport (Björn, 1971). It is suggested that not only PS II, but also part of the cyclic electron pathway and transthylakoid pH gradient are involved in the occurrence of the AG emission which corresponds to a back electron transfer

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towards PS-II centres initially in the S_2/S_3 Q_B state (Sundblad *et al.*, 1988). The occurrence of a flash-induced AG-band may reflect a high level of ATP and/or NADPH in the cells, as shown in *Mesembryanthemum crystallinum* L., a facultative crassulacean-acid-metabolism plant (Krieger *et al.,* 1998).

Crop plants are often exposed to extreme conditions, such as high and low temperature or drought. In this work we show that the AG-band is more sensitive to several abiotic stresses than the B-band, and might be used as a stress indicator in plants.

Materials and Methods

Two-week-old wheat (*Triticum aestivum* L., Cheyenne winter wheat, Chinese Spring spring wheat varieties and Chinese Spring/Cheyenne5A chromosome substitution line) and maize (*Zea mays* L. Norma hybrid) plants grown at 20/18 °C day/night temperature (PPFD = 250 μmol m^{-2} s^{-1}, 16h photoperiod) in a Conviron growth chamber in Hydrokani H nutritive solution were used for the experiments. Cold hardening was carried out at 5 °C at 120 μmol m^{-2} s^{-1} PPFD. Freezing and high temperature treatments were carried out in the sample holder of the TL apparatus.

Thermoluminescence measurements were performed using the laboratory-made apparatus and software described earlier (Miranda and Ducruet, 1995a; Ducruet *et al.,* 1998). This set-up contains a Hamamatsu H5701-50 photomultiplier linked to an amplifier. A 4 × 4 cm Peltier element is used for temperature control. Far-red illumination was provided by a PAM 102-FR light source (Walz, Effeltrich, Germany). The leaf sample was softly pressed against the plate by a Pyrex window, with the addition of 200 μl water for better thermal conduction.

Results and Discussion

Frost

The flash excitation of an unfrozen leaf sample generates a B-band centred at around 35 °C. Freezing at −10 °C had no effect on the maximum temperature in wheat, pea or spinach, although it strongly decreased the B band intensity (not shown). However, in some plant species, such as maize or vine, the B band was downshifted to 20–22 °C after short-term severe freezing. This modification of the B band by freezing was not observed in isolated thylakoids. It can be considered as a freezing artefact, for which a tentative explanation might be the release of vacuolar compounds due to frost-induced membrane disruption.

The far-red illumination of leaves at low but non-freezing temperatures induces a thermoluminescence (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). The $T_{\max}$ of this B-band is lower than the $T_{\max}$ of the B-band induced by one flash, which peaks at around 35 °C. It was shown earlier that increasing the duration of the FR illumination caused a progressive downshift of the B-band (Miranda and Ducruet, 1995a), which can be explained as a destabilization of the S states by progressive lumen acidification (Miranda and Ducruet, 1995b). In control, unfrozen wheat plants grown at 20/18 °C day/night temperature (photosynthetic photon flux density, PPFD = 250 μmol m^{-2} s^{-1}) the $T_{\max}$ of the B-band induced after 30 s far-red light at 0 °C was approx. 15–18 °C, whereas it increased to 28–30 °C after short-term (2 min) freezing at −10 °C (Fig. 1). This partial reversal by frost of the FR-induced downshift of the

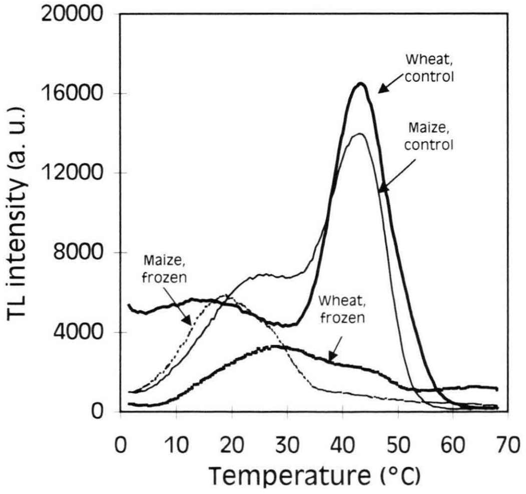

Fig. 1. Effect of 2 min freezing at −10 °C on the far-red-induced TL curve in young maize and wheat plants. TL was induced after more than 8 h dark adaptation by 30 s far-red light at 0 °C.

B-band is similar to that caused by an uncoupler and can be ascribed to freezing damage to the thylakoid membrane (Thomashow, 1998), which leads to a collapse of the proton gradient. It should be noticed that the far-red-induced B band in frozen maize leaves peaked at 22 °C, a location identical to that of the flash-induced B band, which can be considered as a freezing artefact, as discussed above.

Short-term freezing also suppressed the AG-band, usually peaking at around 40–46 °C, in several plant species (Miranda and Ducruet, 1995a). Fig. 1 shows that the AG-band is more sensitive to freezing stress than the B-band. There is usually a sudden drop in the AG-band below a critical freezing temperature. The temperature range in which the AG-band is suddenly lost is within 1 °C. This critical temperature depends on several factors. When the winter wheat variety Cheyenne, which has excellent frost tolerance, was kept at 5 °C for a few weeks (cold hardening) this temperature decreased by approx. 2–3 °C. This hardening effect was less obvious in the variety Chinese Spring (a spring wheat with very poor frost tolerance). When cold-hardened wheat plants were compared, the critical temperature for AG-band suppression was lower not only in the winter variety Cheyenne, but also in the Chinese Spring/Cheyenne5A chromosome susbstitution line than in the spring wheat variety Chinese Spring. Besides 20 chromosomes from Chinese Spring this substitution line contains the 5A chromosome from Cheyenne, which carries the most important gene(s) responsible for the frost tolerance of winter wheat plants (Sutka, 1981).

While in wheat plants a weak AG emission could be seen in frozen samples, in cold-sensitive maize plants this was completely suppressed and only the B-band could be detected (Fig. 1).

Drought / heat

Drought (Fig. 2) and short-term (5 min) heat stress (Fig. 3) affect the far-red-induced TL emission in a similar way to frost, i.e. reversal of the downshifted B band towards higher temperatures and suppression of the AG-band. However, while there was a sudden drop in the AG-band below a critical freezing temperature, an increase in temperature caused a progressive decrease, leading to complete suppression at above 35 °C. Increasing temperature caused a more pronounced decrease in the AG-band (Fig. 3) in wheat (Chinese Spring variety) than in maize plants (Norma hybrid). Heat or drought stress may cause a shift in the $T_{\max}$ of the AG-band towards higher temperatures in maize leaves, which is not observed in wheat leaves. Whilst the harmful effects of heat on PS-II occur above 40 °C, as evidenced by the increase in F_o (Schreiber and Berry, 1977; Havaux *et al.*, 1988), the progressive decrease in the AG band between 30 and 40 °C demonstrates that such temperatures may produce a milder effect on the photosynthetic apparatus, as also evidenced by other methods (Weis, 1984; Moffatt *et al.*, 1990; Havaux, 1993; Galiba *et al.*, 1997).

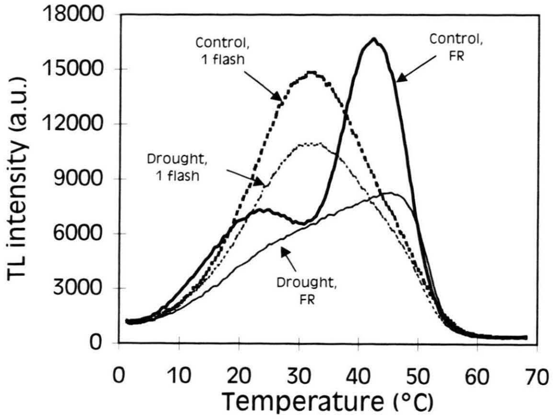

Fig. 2. Effect of 1 d drought on the 30 s far-red (FR) induced TL curve and the B-band induced by 1 flash in young maize plants. Measurements were carried out after more than 8 h dark adaptation. TL was induced at 0 °C.

The mechanisms of the effects of drought or heat on far-red-induced TL emission remain unclear and require further investigation. Their effects (reversal of B-band downshift, decrease in the AG band) are compatible with an increase in membrane proton-leakiness, but other phenomena such as grana destacking above 30 °C (Weis, 1984) should also be considered.

Far-red excitation has two different, although not independent, effects on TL emission :

1. A downshift of the B-band due to lumen acidification, which strongly favours the $S_3 \rightarrow S_2$, and to a lesser extent the $S_2 \rightarrow S_1$ transitions, hence the recombination of Q_B^- with the S_3/S_2 states (there

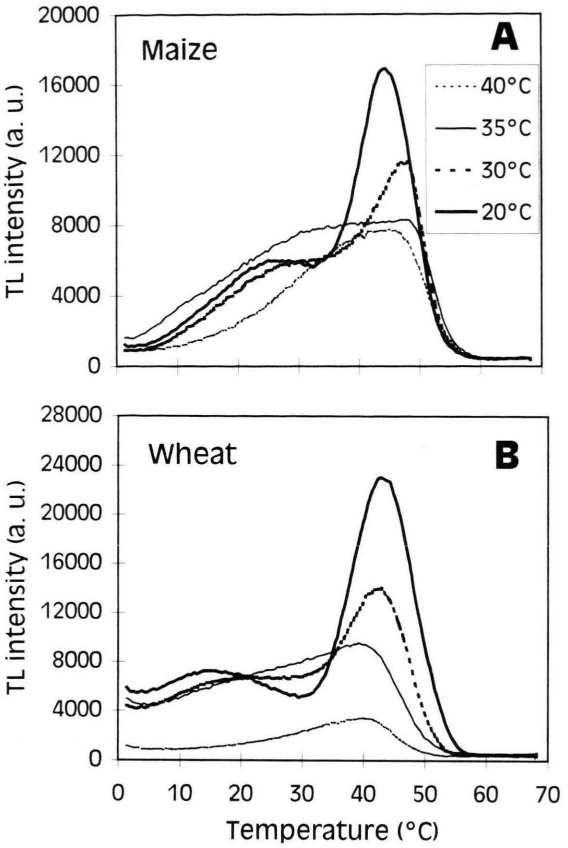

Fig. 3. Effect of 5 min heat treatment on the far-red-induced TL curve in A: young maize (Norma hybrid) and B: wheat (Chinese Spring) plants. TL was induced after more than 8 h dark adaptation by 30 s far-red light at 0 °C.

is always a weak excitation of PS-II by far-red light, which randomizes the S states). Unlike white light, far-red illumination specifically induces a proton gradient, and hence the B-band downshift, without reducing the plastoquinone pool. The reversal of this downshift by uncouplers (Miranda and Ducruet, 1995b), frost, drought and heat demonstrates an increase in the proton leakiness of the thylakoid membrane.

2. The emergence of an afterglow emission, corresponding to an AG-band centred near 45 °C, which also depends on a proton gradient since it is suppressed by uncouplers (Björn, 1971). However, the mechanism of the afterglow emission is a complex phenomenon which requires a reverse electron flow towards the S_2/S_3Q_B centres and involves part of the cyclic electron pathway (Sunblad *et al.*, 1988). In addition to the need for a proton gradient, the AG emission is also likely to be governed by the state of the reducing pool in chloroplasts and by heat-induced structural changes within the thylakoid membranes, which may explain the upshift of the AG-band observed in maize leaves under heat or drought stress. As a consequence, this AG emission appears to be very dependent on several physiological factors (for example the age of the leaf, water content, preillumination), which have to be controlled in order to compare different species or varieties.

These results show that the far-red-induced B and AG-bands are more sensitive to various stress treatments than is the flash-induced B-band and, as a consequence, it could provide a sensitive stress indicator.

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