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THERMOLUMINESCENCE FROM PHOTOSYNTHESIZING SYSTEMS AS A METHOD FOR DETECTION OF EARLY PLANT STRESS SYMPTOMS. EFFECT OF DESICCATION ON THERMOLUMINESCENCE EMISSION PARAMETERS IN MESOPHYTIC AND POIKILOHYDRIC PLANTS

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Summary. The non-invasive optical technique of thermoluminescence (TL) proves to be a simple and valuable procedure in monitoring PSII activity from different photosynthesizing materials, like algae suspension, leaf peaces or isolated thylakoids and oxygen-evolving PSII preparations. The parameters of different bands in TL emission curves are very sensitive to even small changes in the redox potentials of the radical pairs on the donor and acceptor sides of PSII, which makes the obtained information very useful in understanding the mechanisms of injury and preservation of highly sensitive photosynthetic apparatus, namely PSII, in changing environmental conditions. Using TL technique, we observed some peculiarities of PSII redox reactions in resurrection plants, which can reflect specific adaptive characteristics of their photosynthetic system, related to unusual desiccation tolerance of these resurrection plants. In addition to multiple mechanisms for chloroplast integrity preservation, the observed stabilization of charge storage in PSII complex of the resurrection fern *Polypodium polypodioides* L. and the desiccation-tolerant vascular flowering plants *Haberlea rhodopensis* Friv., together with a strong reduction of the total number of PSII centers without any changes in their energetic status, can explain the fast recovery of the photosynthetic activity after desiccation.

Key words: photosynthesis, thermoluminescence, plant stress, desiccation, *Polypodium polypodioides, Haberlea rhodopensis, Spinacia oleracea.*

Abbreviations: HDT – homoiochlorophyllous desiccation tolerant plants; PS II – photosystem II; TL – thermoluminescence.

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INTRODUCTION

Photosynthesis is a basic physiological process determining plant productivity. The functional activity of photosynthetic machinery of the plants brings reliable information on their physiological status and can be used for assessment of the effects of various environmental disturbances or changes, induced by specific mutations or as a result of different selection practices. Researches efforts are concentrated nowadays on the application of sensitive non-invasive optical techniques as a tool for a rapid indirect plant diagnostic. The techniques applied are based on biophysical methods like variable chlorophyll fluorescence and thermoluminescence emission. Integration of the obtained results with the data from the conventional chemical or biochemical analyses permits to receive complementary information about the induced changes.

Thermoluminescence (TL) method provides detailed information on the energetics of photosystem II reaction centers (PSII RC) and gives an opportunity to distinguish the participation of the redox components from the donor or acceptor side of Photosystem II (PSII) complex.

PSII is a multisubunit protein complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria. It uses light energy to catalyze a series of electron transfer reactions resulting in the splitting of water into molecular oxygen, protons and electrons. The initial photon absorption by antenna chlorophyll results in PSII reaction centres excitation. Following charge separation, the electrons from the primary electron donor P680 are passed down and energetically stabilized on the primary and secondary quinone acceptors of the chloroplasts electron transport chain. At room temperature the radical pairs recombine spontaneously leading to re-excitation of P680 and induction of luminescence. Charge recombination can be thermally stimulated and in this case the light emission is called thermoluminescence (Rutherford et al., 1982).

Thermoluminecsence

Photosynthetic TL could be defined as an emission of light at characteristic temperatures from pre-illuminated photosynthetic samples (leaves, isolated chloroplasts or algae) during warming in the dark starting from low temperature (Sane, 2004). A set of different bands in TL emission curves appeare as a result of recombination of different charge pairs. 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.

Thermoluminescence emission from freshly detached unfrozen leaves is defined as secondary or "afterglow" emission with T_m at 45°C and proves to be a sensitive test of energetic imbalance in the chloroplasts during various stress conditions (Ducruet, 2003).

High-temperature thermoluminescence (HT1, HT2 and HT3 bands with T_m above 60°C) appears as a result of accumulation of lipid peroxides and can be used as a simple and efficient tool to monitor oxidative stress in leaves.

The thermoluminescence technique has been used in the Institute of Plant Physiology

since 1995 when a computerized TL set-up (Fig. 1) was constructed (Zeinalov and Maslenkova, 1996). TL has found wide application in the investigations of the Department of Photosynthesis in analyzing PSII structure and function and in understanding the process of plant stress injury and adaptation to heavy metals, salinity, high and low light intensity, desiccation (Maslenkova et al. 1993, Popova et al. 2009; Peeva and Maslenkova, 2004; Georgieva et al., 2003, 2005, 2007).

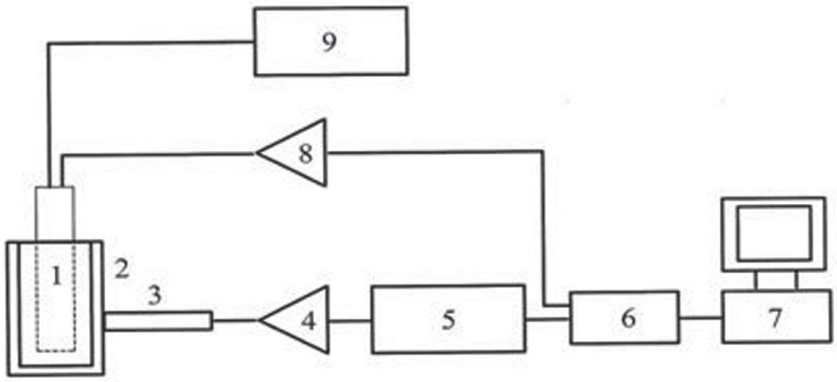

Fig. 1. Thermoluminescence (TL) set-up: 1 – sample holder; 2 – Dewar flask with liquid nitrogen; 3 – photomultiplier tube; 4 – amplifier; 5 – photon-counting device; 6 – ADC plate; 7 – PC; 8 – bridge amplifier; 9 – transformer.

Effect of desiccation on thermoluminescence emmission parameters in mesophytic and poikilohydric plants

Resurrection plants

In our attempt to contribute to the understanding of the mechanisms of desiccation tolerance and sensitivity a comparative analysis of TL glow curve parameters was carried out using representatives of desiccation sensitive (mesophytic) and desiccation-tolerant (poikilohydric) plants.

Desiccation-tolerant or the so-called resurrection plants represent a unique group of organisms able to withstand loss of water to an air-dry state, and to survive extended periods of severe water deficit (Gaff and Hallam, 1974; Bewley, 1979). In a desiccated state their physiological functions, including photosynthetic activity completely cease, but during rehydration this activity can be fully restored, with different rates in homoiochlorophyllous (HDT) and in poikilochlorophyllous (PDT) desiccation-tolerant plants (Tuba et al., 1998).

Various aspects of the desiccation tolerance in vascular plant have received considerable attention, the latest efforts being focused on clearing the physiological and molecular basis of this phenomenon (Gaff, 1997; Ramanjulu and Bartels, 2002; Vicré et al., 2004). However, until now the exact mechanisms preserving the highly sensitive photosynthetic system in the HDT plants during desiccation and the characteristics of the recoverable photosynthetic system in the desiccated stage remain not well understood. The complete reconstitution of chloroplast

structure and functional activity in resurrection plants on rewatering suggests some peculiarities of thylakoid membranes and/or chloroplast stroma composition (Schwab et al., 1989; Maslenkova and Homann, 2000), thus making these plants a very suitable model system for investigation of photosystem II (PSII) complex perturbations and its adaptive plasticity in the course of desiccation and rehydration.

During our studies on the resurrection fern *Polypodium polypodioides* L. (Polypodiaceae) (Fig. 2B) and the desiccation-tolerant vascular flowering plants *Haberlea rhodopensis* Friv. (Gesneriaceae) (Fig. 2A) using a highly sensitive thermoluminescence technique we observed some peculiarities of PSII redox reactions, which can reflect some specific adaptive characteristics of their photosynthetic system, related to desiccation tolerance of these resurrection plants. In addition to multiple mechanisms for chloroplasts integrity preservation, the observed stabilization of charge storage in PSII complex together with a strong reduction of the total number of PSII centers without any changes in their energetic status, can explain the fast recovery of the photosynthetic activity after desiccation.

Polypodium polypodioides and *Haberlea rhodopensis* belong to the group of homoiochlorophyllous desiccation-tolerant (HDT) plants, which upon

Fig. 2. *Haberlea rhodopensis* Friv. (Gesneriacea) (A) and *Polypodium polypodioides* L. (Polypodiaceae) (B) plants in their natural habitat. Morphological changes in the resurrection plants during desiccation (insert).

desiccation preserve above 80% of the chlorophylls and the photosynthetic apparatus is able to recover very fast (Stuart,1968; Markovska et al., 1994; Georgieva et al., 2005). Moreover, the investigated plants have the rare ability for their resurrection to occur in detached leaves (even small leaf pieces).

Leaves from *Polypodium polypodioides* were picked from branches of live oak trees (*Quercus virginiana Mill*.) in Tallahassee (FL), while well hydrated *Haberlea rhodopensis* plants were collected from their natural habitat (the vicinity of Asenovgrad, Bulgaria) during the period of flowering in May. Comparative experiments were done using leaves from the desiccation sensitive mesophytic plant *Spinacia oleraceae* L. (Chenopodiaceae) and leaves from rockcap fern *Polypodium virginianum* L. (Polypodiaceae), collected in near New London, NH. Young, fully expanded leaves, with similar size and appearance were used in the measurements. In order to distinguish the direct effect of water loss on photosynthetic activity and to avoid photoinhibition, the dehydration of detached leaves was carried out in the dark.

Peculiarities of thermoluminescence emission from Polypodium polypodioides and Haberlea rhodopensis leaves

Thermoluminescence (TL) glow curve parameters were used to assess the functioning of both PSII donor and acceptor side components. TL signals have been accepted to result from the thermally activated recombination of the trapped electrons and stabilized positive equivalents on the reduced quinone acceptors (Q_A or Q_B) and on the S_2 (or S_3) oxidation state of the water-splitting complex, respectively. Figure 3 shows TL curves of *Polypodium polypodioides* and *Haberlea rhodopensis* leaves in comparison to those from spinach and from *Polypodium virginianum*. Excitation of dark-adapted spinach leaves with a single flash (F), generating $S_2Q_B^-$ charge pair, induced a B-band peaking at around 32°C (Fig. 3), which was usually observed in the higher plants (Rutherford et al., 1982). The most striking feature of the TL emission observed in the resurrection plant leaves was the up-shift of the B-peak emission temperature to about 45-47°C. Similarly, different emission temperatures were registered when more than one flash had been given (Fig. 3). The B-band position from leaves of *P. virginianum* was about 40°C.

The high emission temperature of the TL B-band from resurrection plant leaves is indicative for more stably stored $S_{2(3)}Q_B^-$ charge pairs in resurrection plants (Rutherford et al., 1984) and could be attributed to some changes in the properties of redox partners on the donor or on acceptor side of PSII, or both. One way to test the contribution of Q_B^- is to monitor TL after infiltration of the leaves with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which specifically inhibits the electron transport between the primary (Q_A) and secondary (Q_B) quinone acceptors. DCMU treatment of spinach leaves leads to a significant downshift in B-band position, concomitantly with a decrease in its amplitude and the appearance of a new, so-called Q-band, peaking at around 0°C (data not shown), which is thought to originate from $S_2Q_A^-$ charge recombination

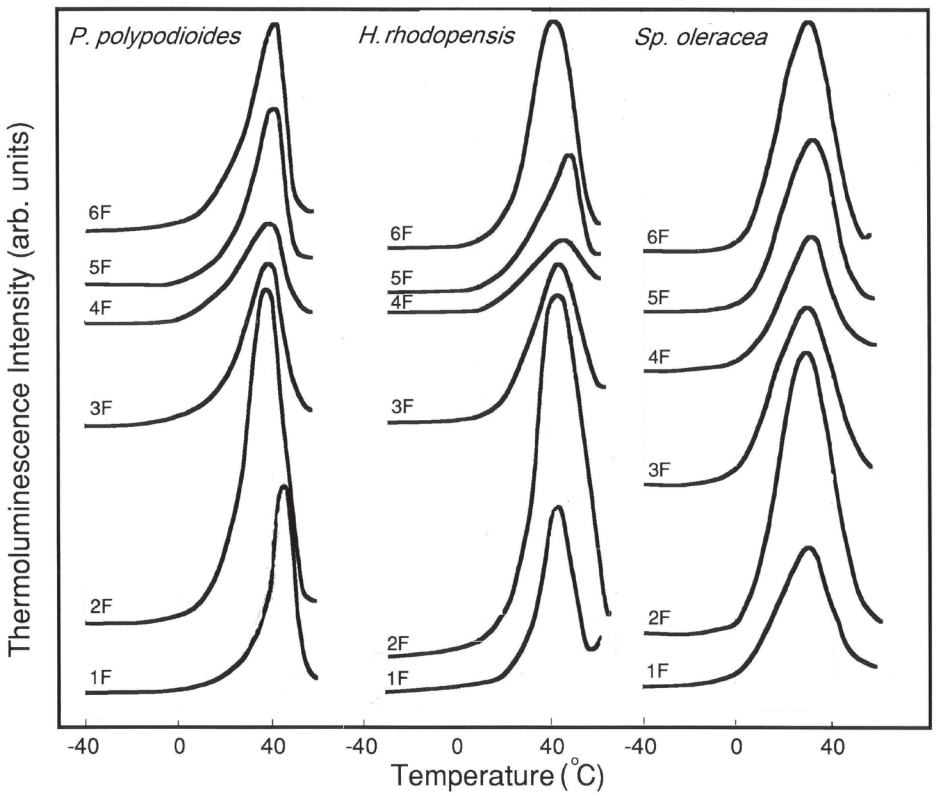

Fig. 3. TL B band emission from fully hydrated dark adapted *Polypodium polypodioides, Haberlea rhodopensis* and spinach leaves excited with one to 6 saturating flashes, given at 5°C. Leaf discs with a diameter 10 mm were used in the experiments.

(Rutherford et al., 1982). In 20 μM DCMU treated *P. polypodioides* (data not shown) and *Haberlea rhodopensis* leaves discs (Fig. 6A 'd') the Q-band also appeared at approximately the same temperature. Since the S-states are the common pool for positive charges of the B- and Q-bands, the distinct differences of B-peak temperature position in resurrection plants and spinach leaves suggest major alterations in the redox property of Q_B^- in the HDT plants. Surprisingly, a part of B-band in *Haberlea rhodopensis* was still clearly expressed even at higher inhibitor concentrations. With the very tough *Polypodium* leaves this dependence was only occasionally observed. These results show that some PSII reaction centers in resurrection plant leaves with more stable stored $S_{2(3)}Q_B^-$ charge pairs are unsusceptible to DCMU, and therefore, can possibly indicate some modifications of the redox properties of the quinone acceptor Q_B (especially in D1 core protein). In accordance with this suggestion are the data of Ohad et al. (1990) and Hideg et al. (1993), considering the observed incomplete suppression of the B-band by DCMU after high light and UV-B irradiation as a proof for acceptor side modifications. Analogical deeper stabilization of $S_{2(3)}Q_B^-$ charge recombination as a result of mutations in D1 protein has also been reported (Mäenpää et al. 1995 ; Alfonso et

al., 1996; Vavilin and Vermaas, 2000; Sane et., 2003). The already described specific lipid and sterol composition of *Haberlea rhodopensis* leaves (Stefanov et al., 1992) and the presence of different protective compounds in chloroplasts stroma may contribute to these modifications. Analysis of the main carbohydrates in the leaves of *Haberlea rhodopensis* and *Polypodium polypodioides* showed sucrose (Muller et al., 1997; Markovska and Kimenov, 1998) and trehalose (Gaff, 1989), respectively, large quantities of which are accumulated during drying.

Changes in thermoluminescence characteristics during dark desiccation and rehydration

Under conditions of severe or prolonged water deficit, most plants are desiccation-intolerant (homoiohydric) and react to stress by suspension of metabolism and irreversible damage to membrane structures and internal organization. Our data of changes in TL B band parameters from dehydrated spinach leaves (Fig. 4) are in agreement with the respective desiccation sensitivity of this

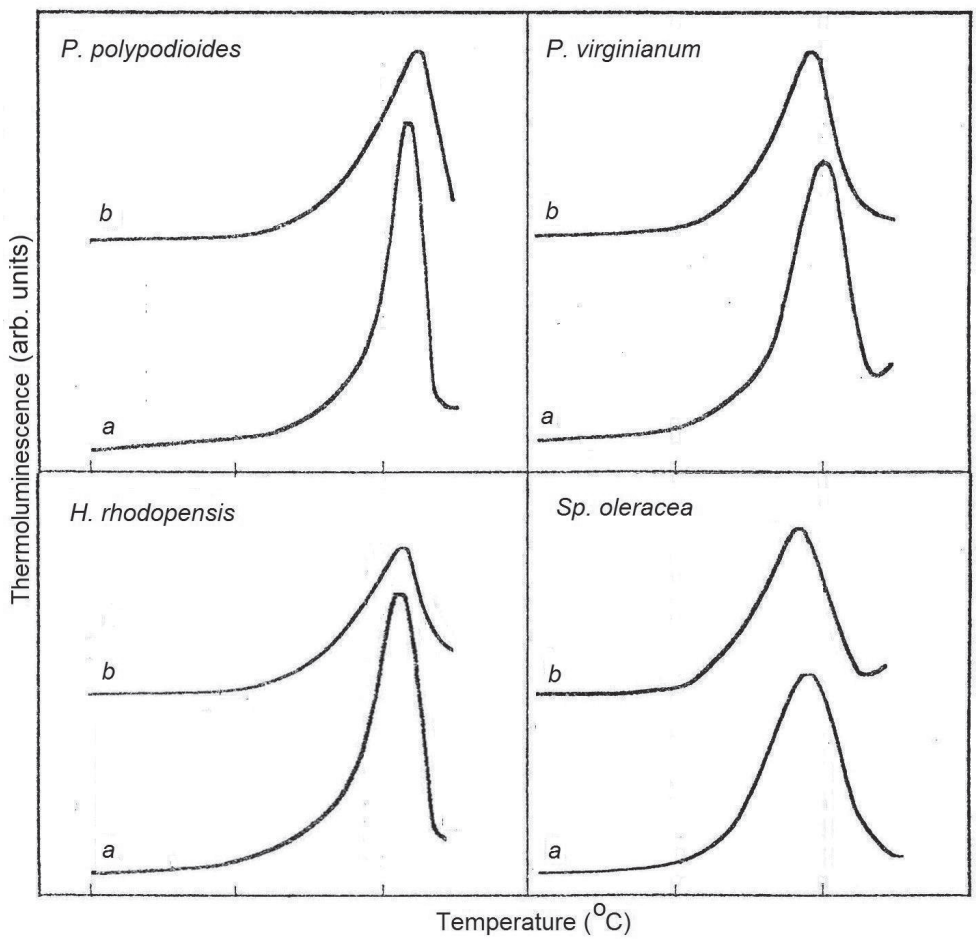

Fig. 4. Effect of dehydration on TL B-band temperature emission. Fully hydrated leaves (a) and leaves dehydrated to 50% RWC in the dark (b).

mesophytic plant. Severe dehydration of the leaves inhibits the number of operating centers, but leads predominantly to a well-expressed down-shift of B-band position close to Q-band position. This observation is indicative for the destabilization of PSII centers as it was also shown in TL study on desiccating barley leaves (Skotnica et al., 2000). It may be concluded that in mesophytic plants subjected to severe dehydration the electron transport between primary (Q_A) and secondary (Q_B) quinone acceptors is inhibited, and damaged oxygen-evolving complexes occurred. Such PSII centers do not restore their photochemical activity during rehydration (Fig. 5). Even more pronounced differences are in agreement with the

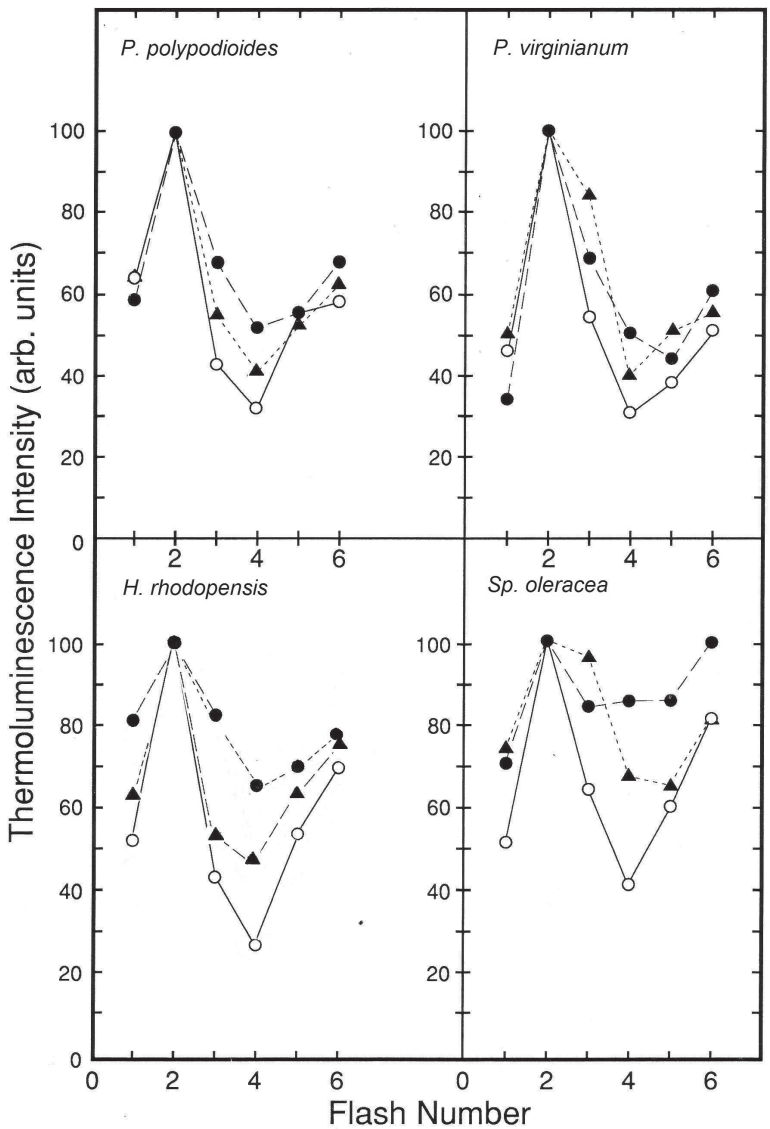

Fig. 5. TL B band oscillations as a function of flash number (1F to 6F, given at 5°C). Fully hydrated leaves (solid line, ○—○); leaves dehydrated to 50% RWC(▲····▲) and leaves rehydrated in moist filter paper for 24 hours (●- - -●). Amplitudes were normalized at the second flash.

results of changes in the so-called stress markers (electrolyte leakage, malondialdehyde, hydrogen peroxide and proline content) (Georgieva et al., 2005), showing that damages sustained during dehydration become particularly detrimental after full metabolic activity has set in with spinach leaves rehydration.

The most important result emerging from thermoluminescence studies of *P. polypodioides* and *Haberlea rhodopensis* leaves is that severe dehydration of resurrection plants affects mainly the number of PSII reaction centers, judging from the significant decrease of B-band amplitude (Fig. 4) without any changes in the energetics state of the remaining operative centers. After rehydration of desiccated leaves the number and the oscillation pattern of operating PSII centers were nearly completely restored (Fig. 5). This process was very rapid and rehydration for only 2 hours restored more than 80% of the initial B-band amplitude.

The effect of desiccation and subsequent rehydration on the redox functioning of PSII donor and acceptor side redox components of *Haberlea rhodopensis* leaves was also assessed by the changes in the main TL bands emitted at illumination with continuous white light during cooling the leaf disks from room temperature to -20°C. Under these experimental conditions a complex glow curve with well-resolved TL bands at about 0°C and 45°C, corresponding to Q- and B-bands (Vass and Govindjee, 1996) was obtained. Representative TL curve pattern from the leaves of fully hydrated *Haberlea rhodopensis* plants is shown in Fig. 6 (‘a’ and ‘b’).

The traces in Fig. 6A (‘e’ to ‘h’) reveal that increasing dehydration resulted in clear changes in the overall intensity of TL signals and in re-distribution of the TL emission between the existing Q and B bands with practically unchanged peak temperatures. In desiccated leaves, the amplitude of the TL B band ($S_{2(3)}Q_B^-$) sharply decreases, and mainly a charge recombination related to $S_2Q_A^-$ (Q-peak) takes place. After rehydration the curve pattern resembles that of the control (fully hydrated) leaves (Fig. 6A, curve ‘h’), which means the electron transport between primary and secondary electron acceptors was reversibly modified. Analogical changes in the amplitude and oscillation pattern of the main TL B- and Q-bands obtained during flash illumination (Fig. 6B), suppose that some changes in the kinetic characteristics of S_2 and S_3 states of PSII donor side during desiccation cannot be excluded.

We suggested that the increased contribution of $S_2Q_A^-$ charge recombination in dehydrated *Haberlea rhodopensis* leaves served to protect Q_B site from over excitation. There are data that the increased population of Q_A^- enhances the probability of non-radioactive energy dissipation and represents an effective protection mechanism (Vavilin and Vermaas. 2000).

Answering the question whether the unique thermoluminescence properties of the chlorophyll molecules of *Polypodium polypodioides* and *Haberlea* leaves are determined by some structural peculiarities required thermoluminescence to be measured on isolated thylakoids. The obtained results show that the isolated photosynthesizing membranes from resurrection plants retain to a great extent the unusual thermoluminescence

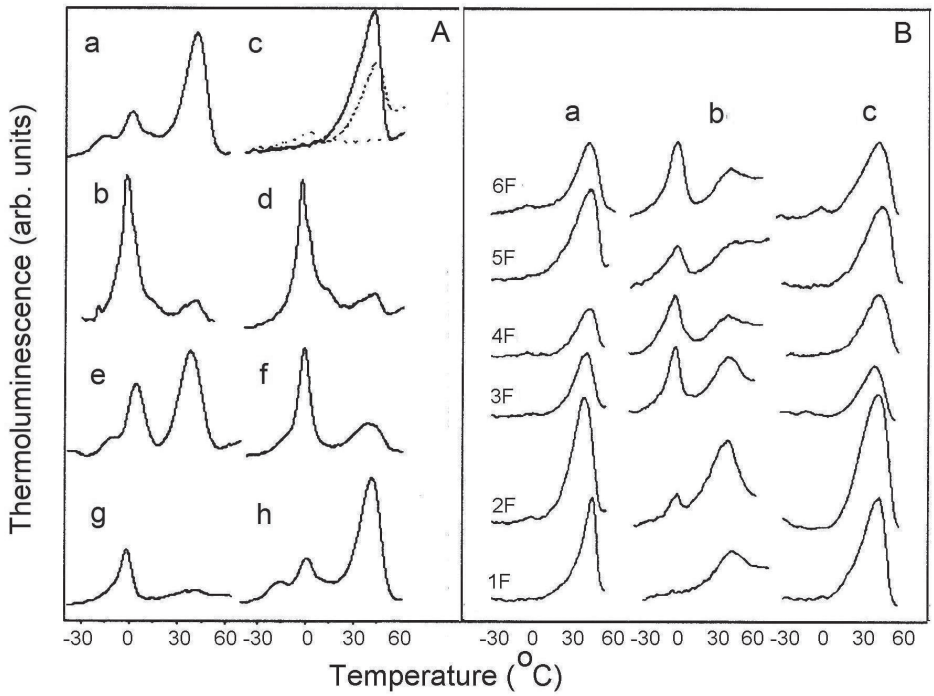

Fig. 6. TL curves of *Haberlea rhodopensis* dark adapted leaves after illumination by continuous white light of 150 μmol m^{-2} s^{-1} from room temperature to -20°C for 1 min (A). Fully hydrated leaves in the absence (a) and in the presence of 20 μM DCMU (*b*). Effect of dehydration on TL signals: control (e), 50% RWC (f), 5% RWC (g) and rehydrated leaves (h).
B-band from control leaves illuminared with 1F (c, dashed line) and after dehydration to 50% RWC (c, dotted line). TL Q-band (d). (B) - TL curves pattern in control (a), dehydrated (b) and rehydrated (c) leaves as a function of the flash number.

pattern of intact leaves, thus indicating the stabilization of S2QB- charge recombination to be an intrinsic feature of PSII complex (Maslenkova, 2009). Moreover, thermoluminescence emission pattern of chloroplasts isolated from desiccated to 20% RWC *Haberlea rhodopensis* leaves was identical to this of chloroplasts isolated from fully hydrated plants, thus indicating their complete rehydration when setting in resuspension medium. The functional activity, including the kinetics of oxygen evolving reactions, is fully recovered in conformity with the preserved membrane integrity. The significant downshift in temperature maximum, the decrease in the intensity of the respective TL bands, and the damping in oxygen yield oscillations in chloroplasts isolated from desiccated leaves of desiccation sensitive spinach are a consequence of the membrane injuries occurred during severe stress.

CONCLUSION

The homoiochlorophyllous resurrection plants *Polypodium polypodioides* L. and *Haberlea rhodopensis* Friv. demonstrated a deeper stabilization of PSII charge pairs, evidenced by an unusually high temperature maximum of the main

thermoluminescence B-peak in leaves and in isolated thylakoid membranes. In addition, a part of these centers was less susceptible to the inhibitor of electron-transport DCMU. These features as well as the strong reduction of the number of active PSII centers performing $S_{2(3)}Q_B^-$ charge separation during desiccation, without any changes in the energetic of charge recombination in the rest operating centers, were considered to indicate some specific adaptive characteristics of the photosynthetic system, related to desiccation tolerance of the HDT fern *Polypodium polypodioides* and vascular flowering plants *Haberlea rhodopensis*. As far as such unusual TL properties have been reported also for desiccation tolerant lichen *Cladonia convulata* (Sass et al., 1996), it is reasonable to suggest that they represent a common protection mechanism of HDT plants during frequent unfavorable changes in water availability in their natural habitat.

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