

Combined effects of Thylakoid Energisation Level and Water Deficit on Thermal Stability of Photosystem II in a Dessication Tolerant Moss

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Abstract. The thermal stability of photosystem II was examined under different (light and water deficit) treatments in the moss *Homalothecium lutescens* (Hedw.) Robins. In the dark-adapted samples, according to the critical temperatures of the F_0 vs. T curves (T_c , T_p , $F_{1/2}$), the temperature tolerance measured in the default state (non-energised thylakoids at full turgor) was inadequate to withstand the thermal conditions of the original habitat. Similarly to some higher plants, the temperature dependence of the steady-state level of fluorescence resulted in a significant increase of thermal stability of PS II at a low excitation energy level ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density). This decrease in heat sensitivity was more expressed at higher levels of excitation energy. Parallely, compared to the optimal quantum yield (F_v/F_m) measured in the dark-adapted state, the heat dependence of the effective quantum yield of PSII ($\Delta F/F_m'$) measured in samples with a steady-state photosynthesis level signalled a higher thermal stability.

The decrease of water content under continuous light effected a similar heat-tolerance increase, further intensified by increasing excitation energy levels. The breakpoints (T_c , T_p , $F_{1/2}$) of the F_s vs. T curves were significantly shifted towards higher temperatures even under a 30-minute moderate (-1.3 MPa) osmotic treatment, which was inhibited by DTT. Both a moderate and a higher water deficit (-2.5 MPa) resulted in an increase of thermal stability,

independent of the excitation energy level. This effect of water deficit remained observable in a longer period (24–48 h). Since in a dark-adapted state the critical values of the F_0 vs. T curves did not shift towards significantly higher temperatures with the increase of water deficit, it seems likely that the water deficit induced rapid thermal stability increase of PSII happens only in energized photosynthetic membranes.

The temperature dependence of non-photochemical quenching and the changes under DTT treatment seem to suggest that the low lumen pH and the related processes might have a role in the protecting mechanisms concerning both heat stress and water deficit: it seems likely the protection against excess light, high temperature, and water deficit reveals common characteristics, in this kryptogam plant, at least.

Keywords: thermal tolerance, photosystem II, water deficit, *Homalothecium lutescens*

Abbreviations used

AL: actinic light; DTT: dithiothreitol; $\Delta F/F_m'$: effective quantum yield of PS II; F_0 , F_s : initial and steady state levels of chlorophyll *a* fluorescence; F_v/F_m : optimal quantum yield of PS II; NPQ: non-photochemical quenching; PS II: photosystem II; T : temperature; T_c : critical temperature; T_p : peak temperature.

Introduction

In natural habitats the different ecological factors (temperature, light, water condition) vary in interdependence with each other. Simultaneous stress factors may elicit a response different from that given to a single factor, resulting in intensification, overlapping or antagonistic effects (Osmond *et al.* 1986).

The heat sensitivity of plants is closely connected to the thermal stability of PSII. It is more or less clear that the heat sensitivity of the photosynthetic apparatus, and the thermal stability of PSII, can change rapidly (within tens of minutes) as a result of heat pre-treatment (Havaux and Tardy 1996). However, in spite of the wide-ranging research in the field (Björkman 1987; Gamon and Pearcy 1990; Havaux 1992), it is still not widely recognized how these short-term responses to heat are influenced by other stress factors like light and water deficit, or desiccation. The study of these problems is further justified by the fact that under natural conditions high light intensity, heat stress, and water deficit occur in combination with each other. A good example for this is that the presence or absence of light can significantly modify the measure of heat-induced damage: the photosynthetic apparatus is

probably more stable in light than in dark (Molnár *et al.* 1998). Besides, the artificially generated intrathylakoid pH gradient effects an accumulation of zeaxanthin and a parallel increase of the thermal stability of thylakoid membranes (Weis 1982, Havaux and Gruszecki 1993). All this raises the possibility that the regulating/protecting mechanisms linked to low lumen pH have a role in the protection against the harmful effects of excess light as well as of heat stress.

Poikilohydric plants rapidly lose water in high light and temperature conditions, and thus a third stress factor needs to be survived here. However, during water deficit the relative water content (Bajji *et al.* 2000) and the activity of some photosynthetic processes decrease (Cheves 1991; Cornic 1994; Cornic and Massacci 1996; Cornic 2000; Lawror 1995) there are observations to the effect that in higher plants the slow dehydration of removed leaves resulted in an increase of the thermal stability of PS II, detected on the basis of the temperature dependence of the initial level (F_0) of chlorophyll a fluorescence (Havaux 1992). Since parallel with the increase of irradiation and leaf temperature the water content rapidly decreases in poikilohydric plants, to achieve an adequate dry matter production and growth rate, an efficient photosynthetic functioning is necessary even under such unfavourable conditions: the effect of the three stress factors needs to be tolerated at the same time, which is impossible without high thermal stability of photosynthetic functions. On the other hand, the thermal tolerance of PSII measured in darkness and at full turgor is probably not adequate for tolerating the temperatures of the original habitat, since during the daytime the leaf temperature may rise as high as 40–45 °C. This short study reports the effects on the thermal stability of the photosynthetic apparatus of increasing light intensity and decreasing water content in the dessication tolerant *Homalothecium lutescens* moss.

Materials and Methods

Plant Materials

All experiments were performed on green segments of *Homalothecium lutescens* (Hedw.) Robins. moss. Mosses were collected from a semidry rocky biotope in NE Hungary in early summer, and were stored in the territory of a weather station under similar exposition and conditions to those in the original biotope or in a greenhouse. Before the measurements the samples were rehydrated and transferred to a growth chamber for two days at 25 °C where mosses were stored under fluorescent illumination with a 16-h photoperiod of white light at a photon flux density of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at 100% relative air humidity. Short-term (30 min) osmotic treatments were

performed using polyethylene glycol solutions with -1.3 and -2.5 MPa osmotic potential. Longer term treatments were carried out by dessicators at given air humidity at least for 24 hours but no more than 48.

Chlorophyll a Fluorescence Measurements

The responses of the *in vivo* chlorophyll a fluorescence to temperature change were measured in dark-adapted leaves with a pulse amplitude modulation fluorometer (PAM 101-103, Walz, Effeltrich, Germany) and recorded with a potentiometric chart recorder (NE-244, EMG, Budapest, Hungary) and a computer as described by Dulai *et al.* (1998). The initial level (F_0) of fluorescence was excited by a weak 650-nm light beam modulated at 1.6 kHz ($0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$). The fluorescence was detected by a PIN S1723 photodiode. The maximal fluorescence level (F_m) of the dark-adapted leaves was induced by a white saturating flash ($7000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 0.8-s duration, provided by a Schott KL-1500 light source. After a lag phase of 120 s, a fluorescence transient of 15-min duration was induced by continuous actinic light (AL) of $100\text{-}1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. To analyse the quenching mechanisms, saturation pulses were triggered at steady state fluorescence level and at given temperature values. The variables and equations for quenching analysis were determined according to van Kooten and Snel (1990). The quantum efficiency of photochemistry was calculated as $\Delta F/F_m'$, as described by Genty *et al.* (1989), and the Stern-Volmer coefficient (NPQ) was also used.

Heat-induced Chlorophyll Fluorescence

For the determination of the breakpoints (T_c , $F_{1/2}$ and T_p) of F_0 vs. T or F_s vs. T curves the method of heat induction of fluorescence was applied as described by Schreiber and Berry (1977). The leaves were dark-adapted for 30 min, and then placed on the thermoelectric module. During heating from 25°C to 60°C at a rate of 1°C min^{-1} , the temperature was monitored by a thermocouple thermometer. Heating for F_s vs. T curves was started when the photosynthesis was steady. T_c , $F_{1/2}$ and T_p were determined from the F_0 or F_s vs. T curves.

Results and Discussion

The sensitivity of the photosynthetic apparatus to heat stress is closely linked to the thermal stability of PSII, which is well characterised by the critical values of the temperature dependence of the initial fluorescence level (F_0) of dark-adapted leaves (Schreiber and Berry 1977; Smillie and Nott 1979; Bilger *et al.* 1984). Some studies have already reported that the

photosynthetic apparatus of pre-illuminated leaves reveals higher thermal stability than the untreated control (Havaux and Gruszeczeki 1993). It has also been reported that in higher plants slow, continuous dehydration increases the thermal stability of PSII (Havaux 1992).

In the original habitat of *H. lutescens* the temperature often rises to about 45 °C, which is always coupled with high irradiation. The data in Table 1 show that the heat tolerance of PSII determined on the basis of the F_0 vs. T curves is not sufficient for tolerating such high temperatures. However, since the recording of the temperature dependence of F_0 is done in dark, it is inadequate for determining the thermal tolerance of samples in a light-adapted state at a steady-state photosynthesis level. Similarly to F_0 , the temperature dependence of F_s is also biphasic, and the breakpoints of the curve – according to recent results – appropriately show the thermal stability of samples with a steady-state photosynthesis level (Molnár *et al.* 1998). In the energized state of tylakoids increasing the intensity of actinic light, compared with the T_c and T_p values of the F_0 vs. T curves (recorded in darkness), the same values of the F_s vs. T curves are shifted towards significantly higher temperatures, indicating the higher thermal tolerance of PSII (Table 1). This shift was inhibited by DTT, and in the untreated samples, parallel with the upwards shift of the critical values, the effective quantum yield values also indicated a decreased heat sensitivity (not shown by data). In connection with this, in higher plants a close connection has been found between the activity of photoprotective mechanisms and the thermal stability of PSII (Molnár *et al.* 1998).

The critical values of the F_0 vs. T curves of untreated leaves and of leaves which received a 30 min. -1.3 , -2.5 MPa osmotic treatment did not show a significant difference. Similarly, in dark-adapted state a longer water deficit does not effect an increase of thermal stability, either (Table 2 and Figures 1, 2).

Table 1 The breakpoints of the F_0 vs. T (in darkness) and of the F_s vs. T curves at different actinic light intensities in green segments of *Homalothecium lutescens* at 100% relative water content (RWC). Heating for the F_0 vs. T curves was started after 30 min dark relaxation and for the F_s vs. T curves was started when the photosynthesis was steady at given AL intensity. The results are means of data from five independent measurements on different segments from different plants.

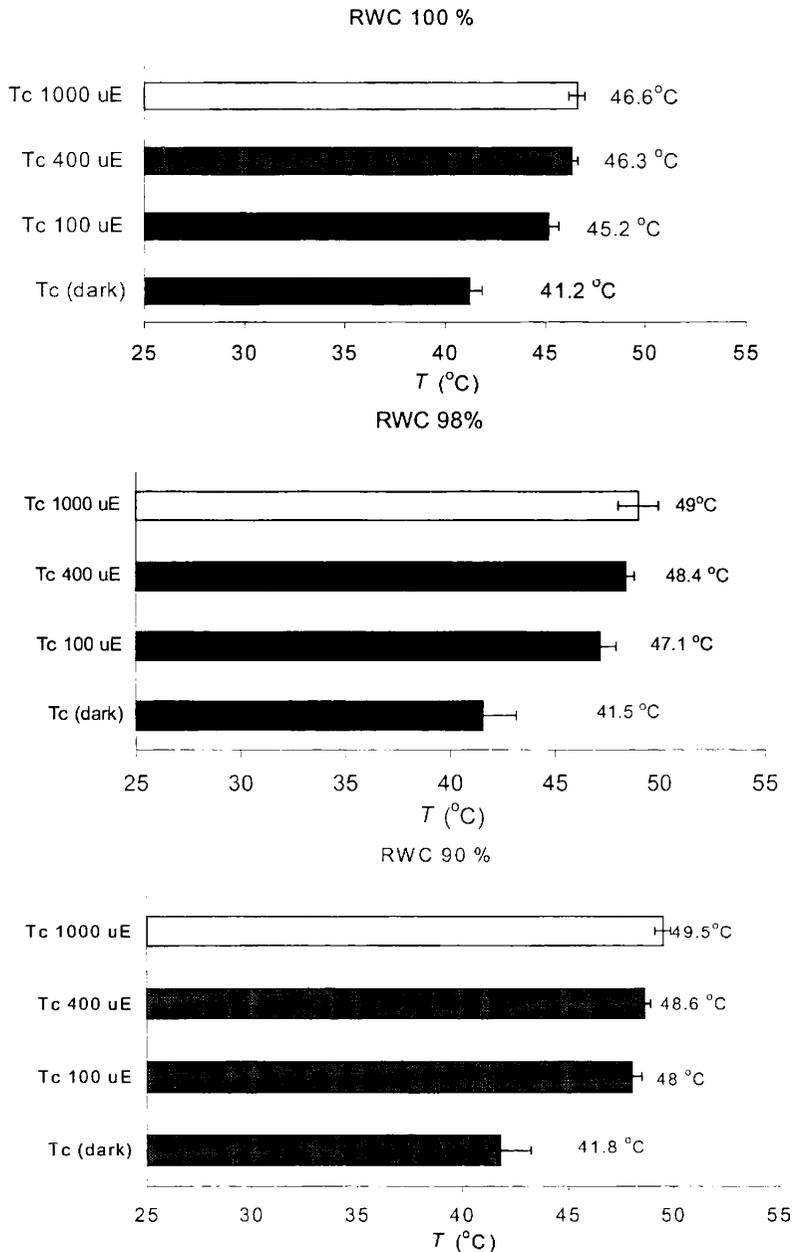
Treatment	T_c	T_p	$F_{1/2}$
Control (dark, F_0 - T)	41.2±0.86	46.1±2.06	43.2±1.6
100 $\mu\text{mol m}^{-2} \text{s}^{-1}$	45.2±0.52	52.1±1.47	47.3±0.87
400 $\mu\text{mol m}^{-2} \text{s}^{-1}$	46.3±0.39	52.3±1.16	48.5±0.94
1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$	46.6±0.49	54.3±0.94	49.3±1.07

Table 2 Effects of the short-term osmotic treatments (-1.3 and -2.5 MPa) and of decreased relative water content (RWC) on the breakpoints of the F_0 vs. T curves (in darkness) in green segments of *Homalothecium lutescens*. Heating for the F_0 vs. T curves was started after 30 min dark relaxation. The results are means of data from five independent measurements on different segments from different plants.

Treatment	T_c	T_p	$F_{1/2}$
Control (100% RWC)	41.2±0.86	46.1±2.06	43.2±1.6
-1.3 MPa (30 min)	41.9±1.23	46.3±1.53	43.5±1.16
-2.5 MPa (30 min)	40.9±1.62	47.1±2.12	44.0±1.82
~98% RWC (24-48 hours)	41.5±1.63	46.9±1.87	43.6±1.82
~90% RWC (24-48 hours)	41.8±1.44	46.0±1.18	42.9±0.97

Table 3 Effects of different short-term osmotic treatments and of different relative water contents on the breakpoints of the F_s vs. T curves in green segments of *Homalothecium lutescens* at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity. Heating for the F_s vs. T curves was started when the photosynthesis was steady at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity. The results are means of data from five independent measurements on different segments from different plants.

Treatment	T_c	T_p	$F_{1/2}$
Control (100% RWC)	45.2±0.52	52.1±1.27	47.3±0.87
-1.3 MPa (30 min)	46.9±1.23	52.9±1.18	49.0±0.72
-2.5 MPa (30 min)	48.1±1.44	54.1±1.01	50.4±0.89
~98% RWC (24-48 hours)	47.1±0.78	55.6±1.87	50.1±1.32
~90% RWC (24-48 hours)	48.0±0.51	56.8±0.94	51.3±1.07



*Fig 1 Effects of increasing actinic light (AL) intensities and of decreasing relative water contents on the critical temperature (T_c) values of the F_0 vs. T and of F_s vs. T curves in green segments of *Homalothecium lutescens*. The results are means of data from five independent measurements on different segments from different plants.*

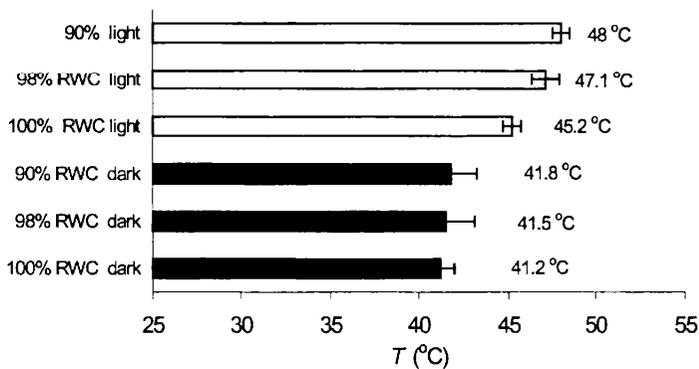


Fig. 2 Effects of decreasing of relative water content (RWC) on the critical temperature (T_c) values of the F_0 vs. T (in darkness) and of the F_s vs. T (at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity) curves in green segments of *Homalothecium lutescens*. The results are means of data from five independent measurements on different segments from different plants.

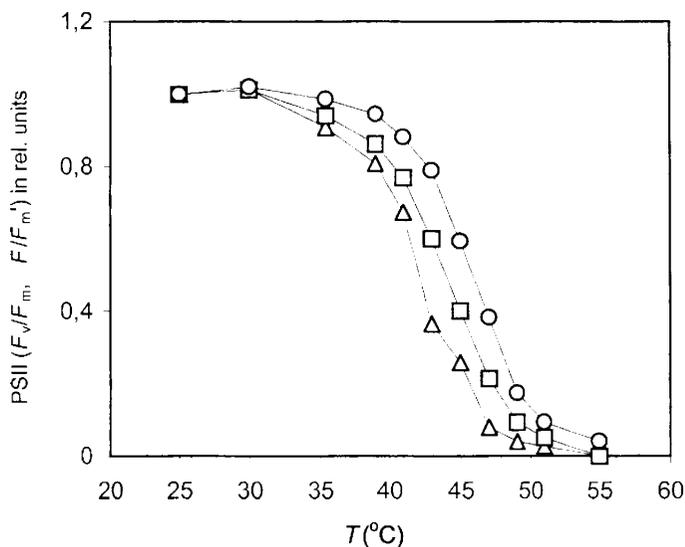


Fig. 3 Effect of decrease of relative water content (RWC) on the temperature dependence of effective quantum yield of PS II ($\Delta F/F_m$) for green segments of *Homalothecium lutescens* at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity (100% RWC, triangles; 98% RWC, squares; 90% RWC, circles). The results are means of data from five independent measurements on different segments from different plants.

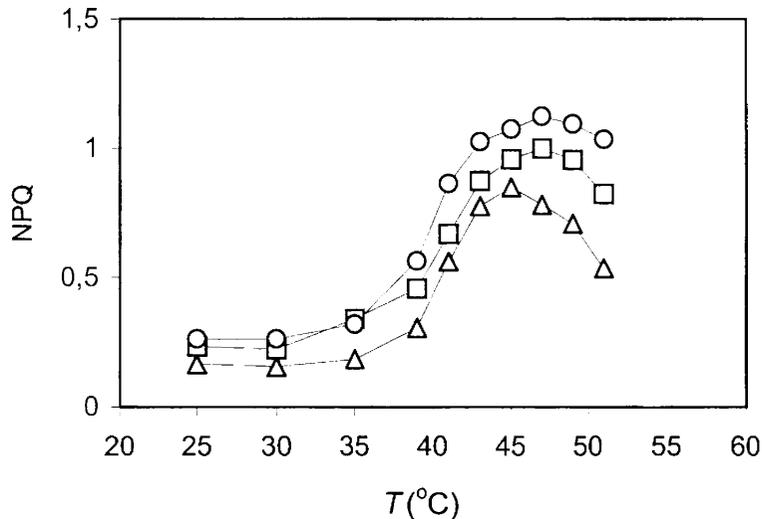


Fig. 4 Effect of decrease of relative water content (RWC) on the temperature dependence of non-photochemical quenching (NPQ) for green segments of *Homalothecium lutescens* at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity (100% RWC, triangles; 98% RWC, squares; 90% RWC, circles). The results are means of data from five independent measurements on different segments from different plants.

As a result of short osmotic treatments, in samples with a steady-state photosynthesis at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ AL intensity the critical values of the F_s vs. T curves were shifted significantly higher, compared to the 100% relative water content control (Table 3), and contrary to some results concerning pea (Dulai *et al.* unpublished data), this increase of heat tolerance prevails under longer water deficit (Figure 2). This enhanced thermal tolerance could have been further increased by the increase of AL (Figure 1). Besides, at given AL intensity greater water deficit also shifts upwards the T_c and T_p values of the F_s vs. T curves, indicating the increased thermal stability of PS II (Figure 1, Table 3). This is also manifested by the temperature dependence of the effective quantum yield of PSII: with the increase of water deficit, the $\Delta F/F_m'$ values start decreasing drastically at higher temperatures (Figure 3). Previous studies have also shown that slow dehydration of intact leaves causes an increase of the thermal stability of PS II, based on the F_0 vs. T curves (in darkness), intensified by the strong pre-illumination (Havaux 1992). Contrary to these results, the rapid osmotic treatment or a longer water deficit at growth light intensity did not cause a significant change in

the temperature dependence of the initial fluorescence level of *H. lutescens*. The increase of thermal stability under water deficit could have been detected only under continuous light (at steady-state photosynthesis level).

The non-radiative dissipation of excess light depends on both the intrathylakoid pH gradient and the activity of the xanthophyll cycle (Demming-Adams 1990). According to some studies, at a low lumen pH the activity of the xanthophyll cycle, besides its role in photoprotection, may have a role to play in the heat tolerance of PS II (Havaux and Tardy 1996; Molnár *et al.* 1998), since the zeaxanthin accumulation, besides intensifying high energy quenching, may increase the thermal stability of PS II with the rigidization of thylakoids (Havaux és Gruszeczki 1993). The NPQ values of treated plants at higher temperatures significantly surpass the control, with maximums close to T_c (Figure 4). Besides, the DTT treatment resulted in a decrease of NPQ values, and inhibited the increase of thermal stability both under light and osmotic treatment (not shown by data). On the other hand, in dark-adapted state water deficit did not result an increase of the thermal stability of PS II (Table 2). All this makes it likely that the thermal tolerance increase caused by water deficit only takes place in energized thylakoids. Therefore it might be possible that the protective processes (in the early stages) against the effects of excess light, high temperatures, and water deficit, at least in this kryptogam species, share certain characteristics, which may be related to the low lumen pH as well.

Acknowledgements

This work was supported by the research grant OTKA T43120. D. S. is grateful to the Hungarian postdoctoral (Békésy György) fellowship.

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