

Water deficit under continuous light enhances the thermal stability of photosystem II in *Homalothecium lutescens* moss

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ABSTRACT The thermal stability of photosystem II was examined under different (light and water deficit) treatments in the moss *H. lutescens*. The decrease in water content under continuous light effected a 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 significantly shifted towards higher temperatures even under a 30-minute moderate (-1.3 MPa) osmotic treatment, and this was partially inhibited by DTT. Both moderate and higher water deficit (-2.5 MPa) resulted in an increase in thermal stability, independent of the excitation energy level. This effect of water deficit remained observable over a fairly long period. Since in a dark-adapted state the critical values of the F_0 vs. T curves did not shift towards significantly higher temperatures with an increase in water deficit, it seems likely that rapid thermal stability increase of PS II induced by water deficit occurs only in energized photosynthetic membranes.

Acta Biol Szeged 46(3-4):159-160 (2002)

The heat sensitivity of plants is closely connected to the thermal stability of PS II. It is more or less clear that the heat sensitivity of the photosynthetic apparatus, and the thermal stability of PS II, can change rapidly (within tens of minutes) as a result of heat pre-treatment (Havaux and Tardy 1996). However, 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 of this is that the presence or absence of light can significantly modify the heat-induced damage: the photosynthetic apparatus is probably more stable in the light than in the dark (Molnár et al. 1998). In addition, the artificially generated intrathylakoid pH gradient effects an accumulation of zeaxanthin and a parallel increase in the thermal stability of thylakoid membranes (Havaux and Gruszecki 1993).

There are observations to the effect that in higher plants the slow dehydration of removed leaves resulted in an increase in the thermal stability, detected on the basis of the temperature dependence of the initial level (F_0) of chlorophyll- α fluorescence (Havaux 1992). Since the water content rapidly decreases in poikilohydric plants parallel with the increase of irradiation and leaf temperature, efficient photosynthesis is necessary even under such unfavourable conditions to achieve adequate dry matter production and growth rate: the effect of the three stress factors needs to be tolerated at the same time. This short study reports on the effects of increasing light intensity and decreasing water content on the thermal stability of the photosynthetic apparatus in the desiccation-tolerant moss *H. lutescens*.

Materials and Methods

All experiments were performed on green segments of

Homalothecium lutescens (Hedw.) Robins moss. Before the measurements the samples were rehydrated and transferred to a growth chamber for two days (25 °C, 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, 100 % RH). 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 using exsiccators at given air humidity for at least 24 hours but for no more than 48.

The responses of the *in vivo* chlorophyll-a fluorescence to heat were measured in dark-adapted leaves with a pulse amplitude modulation fluorometer (PAM 101-103, Walz, Effeltrich, Germany). For the determination of the breakpoints (T_c , $F_{1/2}$ and T_p) of the F_0 vs. T or F_s vs. T curves the heat induction of fluorescence method was applied as described by Schreiber and Berry (1977).

Results and Discussion

In the original habitat of *H. lutescens* the temperature often rises to about 45-50 °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 the toleration of such high temperatures. However, since the temperature dependence of F_0 is recorded in the 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 – provide a satisfactory illustration of the thermal stability of samples with a steady-state photosynthesis level (Molnár et al. 1998). In the energized state of the thylakoids an increase in the intensity of the actinic light caused T_c and T_p values of the F_s vs. T curves to shift towards significantly higher temperatures, compared with the same values of the F_0 vs. T curves (recorded in darkness) indicating the higher thermal tolerance of PS II (Table 1). This shift was inhibited by DTT, and in the untreated samples, parallel with the upwards shift of the

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Table 1 Effects of different water contents (RWC) and of short osmotic treatments on the breakpoints (T_c , T_p , $F_{1/2}$) of the F_0 vs. T (in darkness) and of the F_s vs. T curves at different actinic light (AL) intensities in green segments of *Homalothecium lutescens*.

Treatment	T_c	T_p	$F_{1/2}$
Control (dark, F_0 - T , at 100 % RWC)	41.2±0.86	46.1±2.06	43.2±1.6
100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL (at 100 % RWC)	45.2±0.52	52.1±1.47	47.3±0.87
400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL (at 100 % RWC)	46.3±0.39	52.3±1.16	48.5±0.94
1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL (at 100 % RWC)	46.6±0.49	54.3±0.94	49.3±1.07
-1.3 MPa 30 min (dark, F_0 - T)	41.9±1.23	46.3±1.53	43.5±1.16
-2.5 Mpa 30 min (dark, F_0 - T)	40.9±1.62	47.1±2.12	44.0±1.82
~98 % RWC 24-48 hours (dark, F_0 - T)	41.5±1.63	46.9±1.87	43.6±1.82
~90 % RWC 24-48 hours (dark, F_0 - T)	41.8±1.44	46.0±1.18	42.9±0.97
-1.3 MPa 30 min (at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL)	46.9±1.23	52.9±1.18	49.0±0.72
-2.5 MPa 30 min (at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL)	48.1±1.44	54.1±1.01	50.4±0.89
~98 % RWC 24-48 h (at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL)	47.1±0.78	55.6±1.87	50.1±1.32
~90 % RWC 24-48 h (at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ AL)	48.0±0.51	56.8±0.94	51.3±1.07

critical values, the effective quantum yield values also indicated decreased heat sensitivity (data not shown). In this connection, a close correlation was found in higher plants 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 osmotic treatment at -1.3 or -2.5 MPa did not show a significant difference. Similarly, in the dark-adapted state a longer water deficit did not effect an increase in thermal stability (Table 1). As a result of short osmotic treatments, in samples with 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 shifted to significantly higher values, compared to the 100% relative water content control (Table 1), and, contrary to some results concerning peas (Dulai et al. unpublished data), this increase in heat tolerance prevailed under longer water deficit. This enhanced thermal tolerance was further increased by the increase in AL. In addition, at given AL intensity greater water deficit also shifted the T_c and T_p values of the F_s vs. T curves upwards, indicating the increased thermal stability of PS II (Table 1). This is also manifested by the temperature dependence of the effective quantum yield of PSII: with an increase in the water deficit, the DF/F_m values started decreasing drastically at higher temperatures (data not shown). Previous studies also showed that the slow dehydration of intact leaves caused an increase in the thermal stability of PS II, based on the F_0 vs. T curves (in darkness), which was intensified by strong pre-illumination (Havaux 1992). Contrary to these results, 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 in thermal stability under water deficit was detected only under continuous light (at steady-state the 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 low lumen pH, besides its role in photo-

protection, the activity of the xanthophyll cycle may have a role to play in the heat tolerance of PS II (Havaux and Tardy 1996; Molnár et al. 1998), since zeaxanthin accumulation, besides intensifying high energy quenching, may increase the thermal stability of PS II through the rigidification of the thylakoids (Havaux and Gruszczycki 1993). The NPQ values of treated plants at higher temperatures significantly surpassed the control, with maxima close to T_c . DTT treatment, however, resulted in a decrease in the NPQ values, and inhibited the increase in thermal stability under both light and osmotic treatment (data not shown). On the other hand, in the dark-adapted state a water deficit did not result in an increase in the thermal stability of PS II (Table 1). All this makes it likely that the thermal tolerance increase caused by water deficit only takes place in energized thylakoids. Therefore, it is possible that the protective processes (in the early stages) against the effects of excess light, high temperatures and water deficit, at least in this cryptogamic species, share certain characteristics which may be related to the low lumen pH as well.

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