Photosynth Res
DOI 10.1007/s11120-011-9644-2

REGULAR PAPER

Desiccation of the resurrection plant *Haberlea rhodopensis* at high temperature

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Received: 27 January 2010 / Accepted: 28 February 2011

Abstract *Haberlea rhodopensis* plants, growing under low irradiance in their natural habitat, were desiccated to air-dry state at a similar light intensity (about 30 μmol m^{-2} s^{-1}) under optimal (23/20°C, day/night) or high (38/30°C) temperature. Dehydration of plants at high temperature increased the rate of water loss threefold and had a more detrimental effect than either drought or high temperature alone. Water deficit decreased the photochemical activity of PSII and PSI and the rate of photosynthetic oxygen evolution, and these effects were stronger when desiccation was carried out at 38°C. Some reduction in the amount of the main PSI and PSII proteins was observed especially in severely desiccated *Haberlea* leaves. The results clearly showed that desiccation of the homoiochlorophyllous poikilohydric plant *Haberlea rhodopensis* at high temperature had more damaging effects than desiccation at optimal temperature and in addition recovery was slower. Increased thermal energy dissipation together with higher proline and carotenoid content in the course of desiccation at 38°C compared to desiccation at 23°C probably helped in overcoming the stress.

Keywords Chlorophyll fluorescence · Desiccation-tolerant plant · Photosynthesis · Thylakoid membrane proteins

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Abbreviations

PSII	Photosystem II
PSI	Photosystem I
MDA	Malondialdehyde
RWC	Relative water content
Chl	Chlorophyll
F_v/F_m	Maximal quantum efficiency of photosystem II in the dark adapted state
ΦPSII	Quantum yield of PSII electron transport in the light-adapted state
LNU	Proportion of light not used for photochemistry

Introduction

Desiccation is the most severe form of water deficit when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. Desiccation tolerance includes not only the ability of cells to become air-dry without loss of viability, but also to successfully rehydrate. Only a small group of angiosperms, termed resurrection plants (Gaff 1971), possess desiccation-tolerant vegetative tissues. The physiological basis of desiccation tolerance in resurrection plants is complex and some mechanisms vary among species (Farrant and Sherwin 1998; Bartels 2005). The protective mechanisms include: the production of proteins, sugars and various compatible solutes, which maintain the integrity of macromolecules and membranes; production of antioxidants to minimize free radical damage; subcellular re-organization, such as the folding of cell walls, to minimize mechanical damage associated with turgor loss; dismantling of the photosynthetic apparatus and loss of chlorophyll in poikilochlorophyllous types, and leaf folding and chlorophyll

Published online: 12 March 2011

shading in homoiochlorophyllous types, in order to prevent light-associated damage (Farrant et al. 1999). Desiccation tolerance can be achieved either by mechanisms that are based on the protection of cellular integrity or mechanisms that are based on the repair of desiccation (or rehydration)-induced cellular damage (Bewley and Oliver 1992).

Upon drying, angiosperm tissues must be protected against a number of stresses brought about by, or in association with, extreme water loss. Within their natural habitat, plants are subjected to a combination of different abiotic stresses, each with the potential to exacerbate the damage caused by the others. Recent studies provide evidence that the molecular, biochemical and physiological responses of plants to a combination of abiotic stresses are unique and cannot be directly extrapolated from the responses of plants to each of the stresses applied separately (Barua and Heckathorn 2006). Drought stress is frequently accompanied by high temperatures under field conditions but little is known about how their combination impacts plants (Rizhsky et al. 2004). Of additional concern is the global climate change, which will presumably increase global temperature and intensify drought in arid and semiarid areas.

Haberlea rhodopensis Friv. (Gesneriaceae) is a rare resurrection plant of the northern hemisphere, originating from the Balkan Peninsula as an endemic and relict species of the Tertiary period. From an ecological point of view, *H. rhodopensis* is a perennial, herbaceous, shade-adapted species belonging to the group of homoiochlorophyllous poikilohydric plants which preserve their chlorophyll content during dehydration. It was found that *Haberlea* plants were able to survive desiccation in the dark or at low irradiance (about 30 μmol m^{-2} s^{-1}) under optimal temperature to a water content below 10% with photosynthetic activity fully recovered after rehydration (Georgieva et al. 2005, 2007). Testing the stability of photosystem II (PSII) in leaf disks in high temperature and high light intensities showed its comparatively well expressed thermostability, whereas PSII was very sensitive to photoinhibition (Georgieva and Maslenkova 2006). Dehydration of *Haberlea* in its natural habitat frequently occurs under high temperatures. The objective of this study was to investigate desiccation tolerance of the homoiochlorophyllous poikilohydric plant *Haberlea rhodopensis* under high temperature. The extent of stress treatment was estimated by measuring malondialdehyde (MDA) and proline content and electrolyte leakage. The changes in the efficiency of photosynthetic electron transport and oxygen evolution rate as well as in some proteins involved in the light reactions of photosynthesis were compared upon desiccation of *Haberlea* under optimal or high temperature. To the best of our knowledge, there are no investigations so far on the desiccation of resurrection plants at high temperature.

Materials and methods

Plant material, desiccation, and rehydration

Well-hydrated *Haberlea rhodopensis* plants were collected from their natural habitat where they grow on rocks below trees under very low irradiance. Adult rosettes from the same locality and of similar size and appearance were selected for the experiments. The tufts with naturally occurring thin soil layers were planted in pots in peat-soil. Plants were subjected to drought stress by withholding irrigation either at 23/20°C or 38/30°C day/night temperature, irradiance of 30 μmol m^{-2} s^{-1}, 12 h photoperiod, and relative humidity of 60%. After desiccation to air-dry state, the plants were rehydrated in a reconstructed exsiccator providing permanent humidity by a water pump. Control plants kept at 23/20°C or 38/30°C were regularly watered during the experiment. The measurements were conducted at moderate (RWC of 70 and 50%), strong (15–20% RWC), and severe (4–8% RWC) dehydration as well as after 1 day (R1) and 7 days (R7) of rehydration.

Determination of relative water content (RWC)

The RWC of *Haberlea* leaves was determined gravimetrically by weighing them before and after oven drying at 80°C to a constant mass and expressed as the percentage of water content in dehydrated tissue compared to water-saturated tissues, using the equation:

$$\mathrm{RWC}\ (\%) = \left(m_{\mathrm{fresh}} - m_{\mathrm{dry}}\right) \times 100 \,/\, \left(m_{\mathrm{saturated}} - m_{\mathrm{dry}}\right).$$

Electrolyte leakage

Electrolyte leakage from leaf tissue was measured with a conductivity meter after 24 h incubation of leaf disks (total weight 0.25 g) in 5 ml double-distilled water. The conductivity (μS) of the floating solution was measured and normalized against double-distilled water. Following each measurement, the maximum leakage of the tissue was determined by boiling the leaves 15 min at 100°C. The results are expressed as percentage of maximum leakage.

Measurement of proline and malondialdehyde content

Proline was determined by the method of Bates et al. (1973). 500 mg leaves were homogenized in 6 ml of 3% aqueous sulphosalicylic acid, and the homogenate was centrifuged at 2,000*g* for 5 min. 2 ml of the extract reacted with 2 ml of acidic-ninhydrine and 2 ml of glacial acetic acid for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene. The chromophore containing toluene was separated and the absorbency read at 520 nm.

Malondialdehyde was determined as described by Esterbauer and Cheeseman (1990). 250 mg leaves were homogenized in 3 ml of 0.1% TCA (4°C) and centrifuged at 10,000*g* for 15 min at 4°C. To 0.5 ml of the supernatant, 0.5 ml of 0.1 M TRIS/HCl (pH 7.6) and 1 ml of TCA-TBA-HCl reagent (15% m/v trichloracetic acid; 0.375% m/v thiobarbituric acid; 0.25 M HCl) were added. This solution was boiled 15 min in water bath, centrifuged at 2,000*g* for 5 min, and the absorbance was read at 532 and 600 nm for the determination of malondialdehyde.

Pigment determination

Chlorophyll (Chl) *a*, chlorophyll *b*, and total carotenoids were extracted from leaf disks with 80% acetone. The pigment content was determined spectrophotometrically according to Lichtenthaler (1987), and the data were calculated on a dry weight basis (80°C for 48 h).

Chlorophyll fluorescence

Chlorophyll fluorescence emission from the upper leaf surface was measured with a pulse amplitude modulation fluorometer (PAM 101-103, Walz, Effeltrich, Germany). The initial fluorescence yield in weak modulated light (0.075 µmol m^{-2} s^{-1} PPFD), F_0, and maximum total fluorescence yield emitted during a saturating white light pulse (1 s, over 3,500 µmol m^{-2} s^{-1} PPFD, by Schott KL 1500 light source), F_m, were determined. The leaf disk (10 mm diameter) was then illuminated with continuous red light (100 µmol m^{-2} s^{-1} PPFD). Saturating white light pulses (with 20-s interval) in addition to the background of red light were used to obtain the fluorescence intensity F_m (with all PSII reaction centers closed in any light adapted state). Induction kinetics were registered and analyzed with a program FIP 4.3, written by Tyystjarvi and Karunen (1990).

Re-oxidation of P700

The redox state of P700 was monitored in vivo as 810/860 nm absorption changes. A Walz ED 700DW-E emitter/detector unit was connected to a PAM 101E main control unit (Klughammer and Schreiber 1998). The leaf disk (10 mm) was placed in a chamber originally designed for the fluorescence measuring PAM system (Walz, Effeltrich, Germany). P700 was oxidized by far-red (FR) light from a photodiode (FR-102, Walz, Effeltrich, Germany). Intensity of FR light was 13.4 W m^{-2}. FR light was controlled by the PAM 102 unit and applied via the multibranched fiber optic system. The measurement was carried out in the reflection mode.

Oxygen evolution

Oxygen evolution rate was determined using a leaf disk electrode (Type LD2/2, Hansatech, UK). It was measured at 800 µmol m^{-2} s^{-1} PPFD at saturating CO_2 concentration (provided by a carbonate/bicarbonate buffer).

Thylakoid preparation

All leaves of one *Haberlea* plant were harvested and ground under liquid nitrogen to yield a fine powder. This powder was resuspended in buffer A (50 mM Hepes, pH 7.5, 400 mM NaCl, 10 mM $MgCl_2$, 2 g/l bovine serum albumine, 10 mM dithionite, 4 g/l ascorbate) and centrifuged for 5 min at 8,600*g* and 4°C. Dithionite had to be added to avoid extensive oxidation of phenols, rendering further preparation steps impossible. The supernatant was carefully removed and the pellet resuspended in buffer A. This washing to remove phenol oxidase function was repeated and afterward the sample was filtered through two layers of muslin and cotton wool. The filtrate was centrifuged for further 10 min at 8,600*g* and the pellet resuspended in less than 50 ml buffer B (50 mM Mes, pH 6.0, 150 mM NaCl, 5 mM $MgCl_2$, 1 g/l BSA, 0.5 g/l ascorbate). After a further centrifugation step at 8,600*g* 4°C for 10 min, the pellet was resuspended in 200 µl buffer C (50 mM Mes, pH 6.0, 5 mM $MgCl_2$, 15 mM NaCl) in order to obtain a sufficient concentration of Chl for further separation procedures.

SDS-PAGE and Western blotting of proteins of the PSI and PSII reaction centers, LHC and PsbS

Samples were separated on Tris-Tricin Urea gels according to Schägger and von Jagov (1987). Amounts of solubilized protein equivalent to 15 µg chlorophyll were loaded into each well of 10% (w/v) polyacrylamide gels containing 6 M urea. For Western blots, gels were first equilibrated in cathode buffer (25 mM Tris/HCl, pH 9.4; 40 mM glycine; 10% (v/v) methanol). For transfer of the proteins onto a polyvinyliden fluoride (PVDF) membrane, filter papers soaked in two different anode buffers (0.3 M Tris/HCl, pH 10.4; 10% methanol and 25 mM Tris/HCl, pH 10.4; 10% methanol) and in cathode buffer were used. Transfer was carried out for 60 min, at a current of 1.5 mA cm^{-2}. The membranes were treated with the antisera solutions, the resulting bands visualized by chemoluminescence (ECL kit, Amersham), and signals were recorded on X-ray film. Stripping of the antibodies in order to probe one blot with different antibodies was carried out as recommended by the manufacturer. Antibodies were directed against LHC, PsaA/B (kind gifts of Dr. Jürgen Feierabend, University of Frankfurt), PsbS (a kind gift from Dr. Christiane Funk,

University of Umeå), D2 (a kind gift of Dr. Doris Godde, University of Bochum) and used in a dilution of 1:1000.

Statistical analysis

Control and water stress treatments were statistically compared. Comparison of means from six separate experiments, each in three replications was done by the Student *t* test.

Results

Haberlea rhodopensis plants, growing under low irradiance in their natural habitat, were desiccated to air-dry state at a similar light intensity (about 30 μmol m^{-2} s^{-1}) under optimal (23/20°C) or high (38/30°C) temperature. Dehydration of plants at high temperature increased the rate of water loss threefold. RWC of leaves reached 8% after 24 days or 7 days of drought at 23 and 38°C, respectively. After rewatering, severely dehydrated plants at 23 and 38°C recovered quickly: the RWC of leaves was 80 and 60%, respectively, in 1 day (R1), and reached 95% after 7 days of rehydration (R7).

The RWC of plants, which were regularly watered but exposed to high temperature (control for high temperature treatment), was 85% at a time when dehydrated plants had only 70% RWC and did not change further till the end of the experiment. Moreover, the values of the investigated parameters were very similar in the course of the high temperature treatment. For that reason, the data of the high temperature controls are given as means of the four measurements, each made at the time of the respective degree of dehydration (70, 50, 20, and 8% RWC). Throughout the experiments, leaves of the fully hydrated control plants kept at 23°C showed no significant differences in RWC.

Membrane integrity was assessed by measuring the extent of electrolyte leakage from leaves of control plants and those dried under optimal or high temperature to various RWCs. Upon desiccation of *Haberlea* at 23°C, the electrolyte leakage gradually increased and it was fourfold higher in the dried leaves than in control plants (Fig. 1a). However, after 1 day of rehydration (R1), it sharply decreased and was close to the control after 7 days (R7). The leakage from leaf tissues was enhanced by high temperature treatment, especially when it was combined by drought stress. After 1 day of rehydration (R1), it was still at the same level as in dried leaves (8% RWC) but strongly decreased after 7 days of rehydration (R7).

The changes in the MDA and proline content are generally used as stress markers. Our data showed that MDA content slightly increased as a result of moderate dehydration (70 and 50% RWC) at 23°C ($P < 0.05$, Fig. 1b).

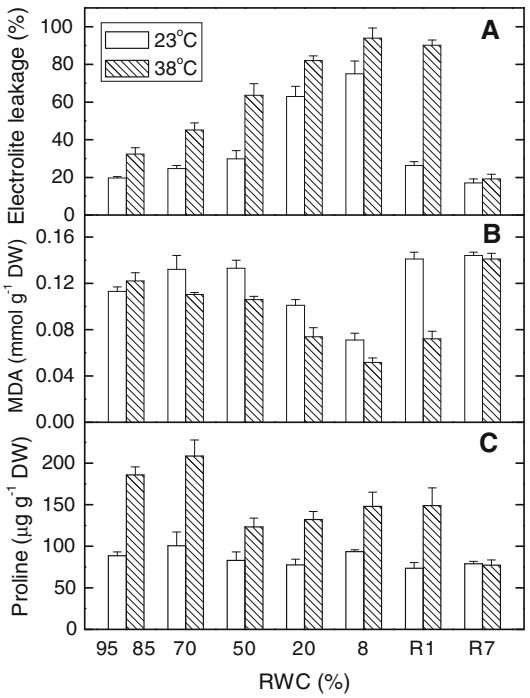

Fig. 1 Changes in electrolyte leakage (**a**), malondialdehyde (**b**), and proline content (**c**) during dehydration as well as after 1 day (stage R1) and 7 days (stage R7) of rehydration of *Haberlea rhodopensis* at optimal (23°C) and high (38°C) temperature under low irradiance (30 μmol m^{-2} s^{-1}). Control plants, kept at 23/20°C (RWC 95%) or 38/30°C (RWC 85%) day/night temperature were regularly watered during the experiment. Mean of six separate experiments, each in three replications with standard error

Then it decreased and was reduced by approximately 40% in severely desiccated leaves ($P < 0.001$; 8% RWC). Following rehydration, MDA content was above the control level. High temperature treatment did not influence MDA content significantly and it was close to the control level under moderate dehydration. Under further water loss, its content declined. After rehydration, MDA content started to increase (R1) and it was above the control value after 7 days (R7).

The proline content was not affected significantly by drought stress under optimal temperature (Fig. 1c). However, exposure of *Haberlea* plants to high temperature increased its content to double and it rose further due to dehydration at 38°C to 70% RWC. The proline content declined with further water loss, but during desiccation and also after 1 day of rehydration (R1), the proline content was still higher than that measured in controls (kept at 23°C) and plants desiccated under optimal temperature. In fully recovered plants (R7), its content was close to the control value.

The chlorophyll content slightly decreased upon desiccation of *Haberlea rhodopensis* under optimal or high temperature, but recovered after rehydration (Fig. 2a). Similar changes were observed in the carotenoid content in

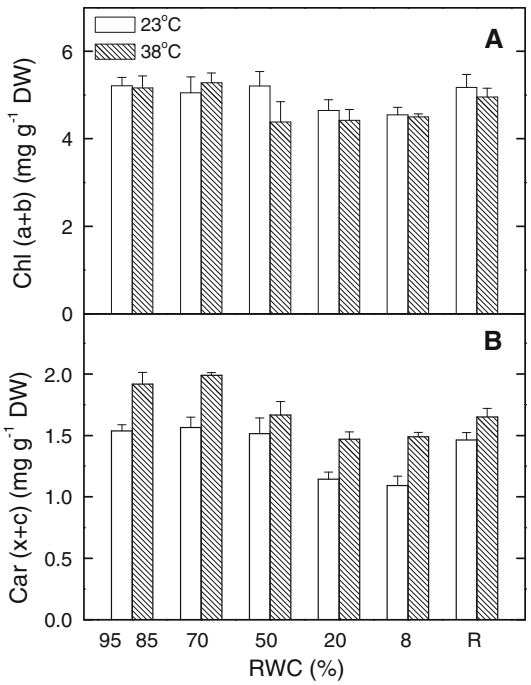

Fig. 2 Changes in chlorophyll (**a**) and carotenoid content (**b**) during dehydration and after 1 day of rehydration (*R*) of *Haberlea* plants at optimal (23°C) and high (38°C) temperature under low irradiance (30 μmol m^{-2} s^{-1}). Mean of six separate experiments, each in three replications with standard error

plants dehydrated at 23°C (Fig. 2b). However, high temperature treatment increased the carotenoid content and it remained higher during drying at 38°C than during desiccation at 23°C.

The maximum quantum efficiency of PSII photochemistry was estimated by the ratio F_v/F_m and F_v/F_0 was used to demonstrate the impact of changes in F_0 (Fig. 3a, b). F_v/F_m gradually declined with decreasing the RWC up to 50%, which was mainly due to an increased ground Chl fluorescence, F_0 (from 0.0344 ± 0.009 at 95% RWC to 0.0518 ± 0.003 at 50% RWC), whereas the maximum Chl fluorescence, F_m, did not change significantly. Further water loss led to a significant reduction of F_m and the ratio F_v/F_m was reduced by 50% at 20% RWC ($P < 0.001$; Fig. 3a). The more sensitive ratio F_v/F_0 decreased by 40 and 60% when RWC dropped to 50 and 20%, respectively (Fig. 3b). The actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) was affected more strongly by both high temperature treatment and desiccation of plants than the ratio F_v/F_m (Fig. 3c). Exposure of plants to 38°C increased F_0 by 30% and lowered F_m by 14% and as a result the ratio F_v/F_m and F_v/F_0 were reduced by 20 and 30%, respectively. High temperature treatment also caused a reduction of ΦPSII by 35%. Desiccation of *Haberlea* plants at high temperature reduced the photochemical activity of PSII much more severely compared to dehydration at optimal temperature and it was due to both higher F_0 and lower F_m values. However, the PSII activity quickly recovered after rehydration. The results showed that the proportion of light not used for photochemistry (LNU, Cornic, 1994) increased in the course of desiccation at 23°C and it was sixfold above the control at 20% RWC (Fig. 3d). LNU values measured in plants desiccated at 38°C were higher than those in plants desiccated at 23°C. After rehydration, LNU decreased but it was still above the control level.

PSI activity, measured by far-red induced P700 oxidation, increased in moderately dehydrated *Haberlea* leaves (RWC up to 50%) but was strongly reduced when RWC dropped to 20% (Fig. 4). Exposure of plants to high

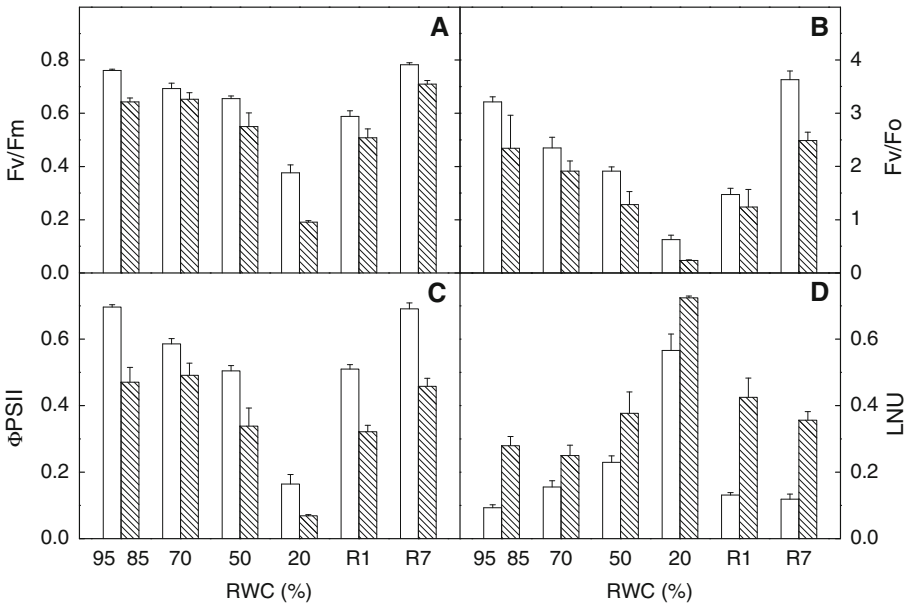

Fig. 3 Changes in maximum quantum efficiency of PSII (F_v/F_m and F_v/F_0), the actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) and the proportion of light not used for photochemistry (LNU) during dehydration as well as after 1 day (stage R1) and 7 days (stage R7) of rehydration of *Haberlea rhodopensis* at optimal (23°C) and high (38°C) temperature under low irradiance (30 μmol m^{-2} s^{-1}). Mean of six separate experiments, each in three replications with standard error

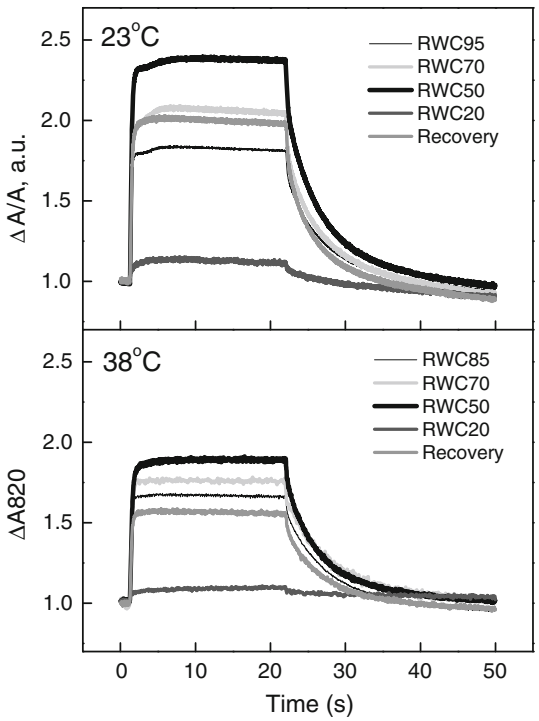

Fig. 4 Changes in the original traces of P700 oxidation induced by 20 s far-red light during dehydration and after one day of rehydration (*R*) of *Haberlea* plants at optimal (23°C) and high (38°C) temperature under low irradiance (30 μmol m^{-2} s^{-1})

temperature slightly decreased the PSI function. Similar to PSII, PSI activity was quickly recovered after rehydration.

The photosynthetic oxygen evolution turned out to be very sensitive to desiccation (Fig. 5). It decreased by 30% already at 70% RWC, and no O_2 evolution was detectable at 8% RWC. High temperature treatment decreased the rate of O_2 evolution by 35% and it was more efficiently reduced when plants were desiccated at 38°C compared to those left at 23°C. After 1 day of rehydration (R1), the rate of O_2 evolution started to increase, with a much faster and better recovery in plants desiccated at optimal temperature. O_2 evolution was close to the respective controls after 7 days of rehydration.

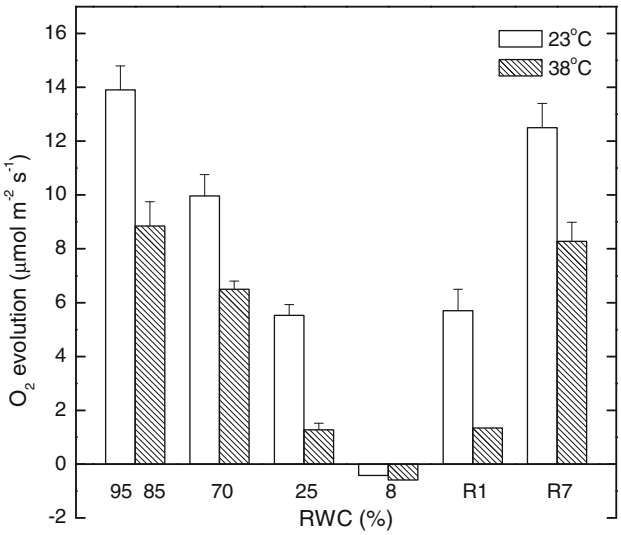

Fig. 5 Changes in the rate of photosynthetic oxygen evolution during dehydration as well as after 1 day (stage R1) and 7 days (stage R7) of rehydration of *Haberlea rhodopensis* at optimal (23°C) and high (38°C) temperature under low irradiance (30 μmol m^{-2} s^{-1}). Mean of six separate experiments, each in three replications with standard error

Using specific antibodies, we investigated the changes in some proteins involved in the light reactions of photosynthesis. We found some reduction in D2, PsaA/B, and PsbS proteins, especially in severely desiccated *Haberlea* leaves (4% RWC) at 23°C (Fig. 6a). During rehydration, their amount started to recover. Concerning the LHC proteins, an increase in desiccated plants was detected. Exposure of plants to high temperature (85% RWC) did not significantly affect the investigated proteins (Fig. 6b). During dehydration at 38°C, they were influenced similarly as compared to 23°C. PsbS seemed to be the most sensitive to desiccation under high temperature. However, its amount significantly increased after rehydration.

Discussion

Effect of high temperature

Among all cell functions, the photosynthetic activity of chloroplasts is believed to be one of the most heat sensitive processes. PSII and Rubisco activase activity are proposed as primary targets of heat stress, and the oxygen evolving complex plays a key role in plant response to high temperature (Havaux and Tardy 1999; Feller et al. 1998). Our results showed that exposure of *Haberlea rhodopensis* to 38/30°C day/night temperature for a week decreased the maximum quantum efficiency of PSII (F_v/F_m; F_v/F_0) and especially the quantum yield of whole-chain electron transfer (ΦPSII, Fig. 3). Similar to ΦPSII, the rate of photosynthetic oxygen evolution was reduced by about 35% (Fig. 5). High temperature treatment reduced PSI activity only slightly (by 8%), confirming the previous results that PSI is more heat resistant than PSII (Sayed et al. 1989; Boucher and Carpentier 1993). However, we found a significant enhancement (about 65%) of electrolyte leakage from leaf tissue indicating some membrane damage (Fig. 1). No significant changes in MDA content could be measured upon heat treatment, although lipid peroxidation is commonly regarded as an indicator of oxidative stress. However, we exposed plants to high temperature at very low light irradiance (about 30 μmol m^{-2} s^{-1}) thus probably limiting oxidative damage. Moreover, the proline content increased twofold after heat treatment (Fig. 1). It was inferred that proline acts as a free radical scavenger

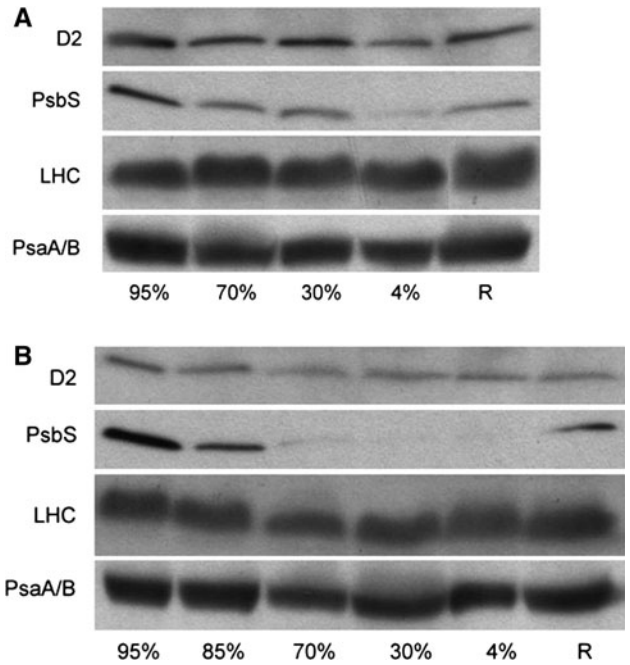

Fig. 6 Immunoblots of thylakoids of control, desiccated at optimal (23°C, **a**) and high (38°C, **b**) temperature and rehydrated (*R*) plants. The antibodies were directed against the proteins indicated on the left. Sample equivalent to 15 μg Chl *a* was loaded in each case for the gel electrophoresis prior to western blotting

and may be more important in overcoming stress than in acting as a simple osmolyte (Reddy et al. 2004). Thus, proline might have helped to avoid oxidative damage as well.

Thermal dissipation of excess energy together with changes in overall photosynthetic capacity can help to balance light absorption and utilization. High temperature treatment of *Haberlea* plants increased the carotenoid content (Fig. 2) and LNU (Fig. 3). Carotenoids can dissipate excess energy as heat via non-photochemical quenching thus preventing formation of singlet oxygen (Frank et al. 1999). All these protective mechanisms help plants to limit the stress induced damage. As a result, the amount of investigated PSI and PSII proteins was not affected by high temperature (Fig. 6), albeit psbS levels were reduced.

Desiccation at high temperature

The present results showed that desiccation of *Haberlea rhodopensis* at high temperature had a more detrimental effect than either drought or high temperature alone. The electrolyte leakage increased during desiccation but it was higher when drought was combined with high temperature, indicating stronger membrane damage (Fig. 1). After 24 h of rehydration, the electrolyte leakage of plants desiccated at 23°C returned almost to control level. Thus, drying appeared to cause some change in membrane configuration, but this was reversed and repaired on rehydration. However, the recovery of plants desiccated at high temperature was slower and the electrolyte leakage significantly decreased after 7 days. According to Oliver et al. (2000) electrolyte leakage upon rehydration is also affected by the speed at which desiccation occurs. As it was mentioned, desiccation at 38°C was three times more rapid than at 23°C.

For many plant species, proline accumulation during stress is an established fact that can be considered an indicative response to stress at the cellular level. Proline has been suggested to function as a mediator of osmotic adjustment, as a stabilizer of macromolecules, as a compatible solute to protect enzymes and as storage for carbon and nitrogen to be used during water deficit and other stress regimes (Ashraf and Foolad 2007). Proline is also known to be involved in reducing the photodamage in the thylakoid membranes by scavenging and/or reducing the production of singlet oxygen. Proline content did not change significantly during desiccation at optimal temperature (Fig. 1). This might be a consequence of a delicate balance of the enzyme activities of the biosynthetic and degradative pathways which is only changed when a second stress like high temperature is applied. Zivkovic et al. (2005) found some dynamic of the proline content during desiccation of *Ramonda serbica* (Gesneriacea), but its amount in desiccated leaves was lower than in well-hydrated ones.

We observed that the proline content was higher in the course of desiccation at 38°C than at 23°C. Probably, this is a marker for the higher extent of stress treatment but the high proline content can as well also be important in overcoming the stress.

Water deficit decreased the photochemical activity of PSII and PSI (Figs. 3, 4), and the rate of photosynthetic oxygen evolution (Fig. 5) and these effects were stronger when desiccation was carried out at 38°C. The decline in PSII activity could represent a protective mechanism from toxic oxygen production in order to maintain membrane integrity and to ensure protoplast survival (Di Blasi et al. 1998). Additionally, the decline observed in F_v/F_m and F_v/F_0 can be rather a regulatory adjustment to limiting carbon availability, imposed by water stress (Saccardy et al. 1998). On the other hand, an increase in the oxidized form of P700 was observed in moderately dehydrated *Haberlea* leaves (Fig. 4). A similar result was also reported by Drozdova et al. (2004) in dehydrated cucumber plants. One possible explanation could be that the limitation of electron flow to PSI caused an increase in P700 oxidation (decreased PSII electron transport activity quantified by the decrease in ΦPSII, Fig. 3). Besides, alterations in the optical properties of the desiccated leaves leading to enhanced P700 oxidation cannot be ruled out.

Haberlea rhodopensis belongs to the group of so-called homoiochlorophyllous species which maintain most chlorophylls in the dried state (Fig. 2). However, this ability requires that the photosynthetic apparatus is maintained in a recoverable form throughout the entire period of dryness. Our previous results showed that the amounts of chlorophyll–protein complexes remain stable during desiccation and rehydration of *Haberlea* (Georgieva et al. 2007), whereas those of desiccation-sensitive plants are completely destroyed after a short-term desiccation event (Lu and Zhang 1999). The present results showed some reduction in the amount of the main PSI and PSII proteins, especially in severely desiccated *Haberlea* leaves (Fig. 6), but their content fully recovered after rehydration.

In summary, our results clearly showed that desiccation of *Haberlea rhodopensis* at high temperature had more damaging effects than desiccation at optimal temperature and that recovery was slower. But the damage was limited to a level where repair was still possible and thus plants fully recovered after 7 days of rehydration.

Acknowledgments This work was supported by the National Science Fund [Project D002-208/2008] and the Deutsche Forschungsgemeinschaft (Bu812/6-1).

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