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ORIGINAL ARTICLE

Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during dehydration and rehydration

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Abstract The functional state of the photosynthetic apparatus of flowering homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during dehydration and subsequent rehydration was investigated in order to characterize some of the mechanisms by which resurrection plants survive drought stress. The changes in the CO_2 assimilation rate, chlorophyll fluorescence parameters, thermoluminescence, fluorescence imaging and electrophoretic characteristics of the chloroplast proteins were measured in control, moderately dehydrated (50% water content), desiccated (5% water content) and rehydrated plants. During the first phase of desiccation the net CO_2 assimilation decline was influenced by stomatal closure. Further lowering of net CO_2 assimilation was caused by both the decrease in stomatal conductance and in the photochemical activity of photosystem II. Severe dehydration caused inhibition of quantum yield of PSII electron transport, disappearance of thermoluminescence B band and mainly charge recombination related to $S_2Q_A^-$ takes place. The blue and green fluorescence emission in desiccated leaves strongly increased. It could be suggested that unchanged chlorophyll content and amounts of chlorophyll–proteins, reversible modifications in PSII electron transport and enhanced probability for non-radiative energy dissipation as well as increased polyphenolic synthesis during desiccation of *Haberlea* contribute to drought resistance and fast recovery after rehydration.

Keywords Blue-green fluorescence · Chlorophyll fluorescence · Chlorophyll–proteins · Desiccation tolerant plant · Photosynthesis · Thermoluminescence

Abbreviations

Ca	Ambient air CO_2 concentration
Chl	Chlorophyll
Ci	Intercellular CO_2 concentration
F_v/F_m	Maximal quantum efficiency of photosystem II in the dark adapted state
gs	Stomatal conductance
LHCI	Light harvesting complex I
LHCII	Light harvesting complex II
NPQ	Non-photochemical quenching
PSI	Photosystem I
PSII	Photosystem II
ΦPSII	Quantum yield of photosystem II photochemistry in the light adapted state
WC	Water content

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Introduction

The desiccation-tolerant or resurrection plants can survive drying to a point where no liquid phase remains in

the cell and the water content may be no more than 5–10% dry weight. Upon watering the plants rapidly revive and are restored to their former state. Unlike other plant responses to drought stress, desiccation in resurrection plants prevents growth and reproduction over the dehydrated period and the plant is preserved until water becomes available (Scott 2000). In vascular plants desiccation tolerance of the vegetative tissue has been demonstrated in only 350 species, making up less than 0.2% of the total flora (Proctor and Tuba 2002). Desiccation-tolerant plants may be subdivided into homoiochlorophyllous and poikilochlorophyllous. The homoiochlorophyllous desiccation-tolerant plants retain their chlorophyll (Chl) during desiccation, whereas in poikilochlorophyllous desiccation results in the loss of Chl which must be resynthesized following rehydration (Tuba et al. 1998). Because of their large size and greater complexity desiccation-tolerant vascular plants tend to dry out and rehydrate more slowly than bryophytes or lichens. Numerous metabolic changes have been found to occur in resurrection plants as they dehydrate and rehydrate. Upon water loss, the decrease in cellular volume causes crowding of cytoplasmic components and the cell contents become increasingly viscous, increasing the chance for molecular interactions that can cause protein denaturation and membrane fusion (Hoekstra et al. 2001). For model membrane and protein systems, a broad range of compound have been identified that can prevent such adverse molecular interactions, among them proline, glutamate, glycine–betaine, mannitol, sorbitol, fructans trehalose, sucrose and oligosaccharides. The sugars can act to stabilize membranes and proteins in the dry state by maintaining hydrogen bonding within and between macromolecules (Alisson et al. 1999) and sugars could vitrify the cell content and stabilize internal cell structure (Crowe et al. 1996). The accumulation of some late embryogenesis abundant proteins and small desiccation-induced proteins to high concentrations coincides with the acquisition of desiccation tolerance and they are thought to play a role in desiccation tolerance (Alamillo and Bartels 2001; Hoekstra et al. 2001). However, when water dissipates from the water shell of macromolecules at a moisture content <0.3 g H_2O g^{-1} dry weight, the hydrophobic effect responsible for structure and function is lost. It is envisaged that sugars, especially the non-reducing disaccharides but also tri- and tetrasaccharides and fructans that accumulate in anhydrobiotes, can replace the dissipating water (Hoekstra et al. 2001).

The mechanisms enabling resurrection plants to survive desiccation are not yet fully understood. The protective mechanisms against desiccation in angiosperms are complex and vary among species (Farrant and Sherwin 1998). A minority of species have been studied in any detail, with most physiological and biochemical work being performed on the genus *Craterostigma*. Therefore, more research is required on a greater range of species to appreciate common elements that exist.

Haberlea rhodopensis Friv. is a perennial herbaceous rock poikilohydric plant, forming dense tufts of leaves, every rosette bearing in spring one to five flower-stalks, each with two to four blossoms. *H. rhodopensis* is a preglacial relict, whose "age" is probably over two million years. Spread all over Europe in the past times, nowadays it can be found only in the Balkan Mountains. It prefers the shady mostly northward slopes of limestone ridges in mountain zones with higher humidity. It is considered as a homoiochlorophyllous desiccation-tolerant plant, since it preserves its Chl content during dehydration. The adult rosettes can dehydrate to water content below 10% and remain in this viable but desiccated state for a considerable time.

The investigations on the mechanisms of desiccation tolerance in *H. rhodopensis* are still scarce. Our previous study was performed on detached leaves subjected to rapid desiccation in the dark (Georgieva et al. 2005). The data showed a strong reduction of the total number of PSII centers in *Haberlea* during desiccation without any changes in the energetic of charge recombination, suggesting the involvement of a protective mechanism related to desiccation tolerance that could be specific for this resurrection species.

In the present paper, we extend our earlier studies by investigating the functional state of the photosynthetic apparatus of *H. rhodopensis* during dehydration of whole plants at low light intensity, i.e. under conditions similar to the natural. Furthermore, we investigated not only changes in PSII activity, but also in the CO_2 assimilation rate and electrophoretic characteristics of the chloroplast proteins. The fluorescence imaging system was used to show variations of the fluorescence signal in blue and green spectral regions as criteria for polyphenolic synthesis. In addition, the recovery of photosynthetic activity following desiccation was also studied.

Materials and methods

Plant material, desiccation and rehydration

Well-hydrated *H. rhodopensis* plants were collected from their natural habitat (the vicinity of Asenovgrad,

Bulgaria) at the period of flowering in May. 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 transferred in a chamber at 22–23°C, light intensity of 30 μmol m^{-2} s^{-1}, relative humidity of 60% and they were allowed to acclimate to these conditions for a week. Plants were subjected to drought stress by ceasing watering and the leaves were fully desiccated after 7 days. Then the severely dehydrated plants were rehydrated for 7 days by spraying water on the leaves to simulate rainfall and keeping the soil damp. The measurements were conducted at middle stage of water loss, i.e. 50% reduction in water content (stage D1; WC 1.51 g H_2O g^{-1}DW) and when the water content was 5% of the control (stage D2; WC 0.16 g H_2O g^{-1}DW).

Determination of pigment content

Chlorophyll *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 leaf dry mass (80°C for 48 h).

Measurements of CO_2 gas exchange rates

CO_2 exchange rates in light (light saturated net CO_2 assimilation or CO_2 release rate) was measured using a CIRAS-2 type IRGA system, operated in differential mode at 700 μmol m^{-2} s^{-1} light intensity at ambient CO_2 concentration of 360 ppm and at 25°C air temperature as described by Tuba et al. (1994). The CO_2 gas exchange parameters (1) CO_2 assimilation, (2) intercellular CO_2 concentration (Ci) and (3) stomatal conductance (gs) were calculated according to von Caemmerer and Farquhar (1981).

Chlorophyll fluorescence

Fluorescence induction measurements of leaf samples were performed using a PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). Leaf discs were placed into a leaf section chamber and dark-adapted for 20 min. F_0 level of fluorescence was determined by switching on the measuring light (modulation frequency of 1.6 kHz and PPFD less than 1 μmol m^{-2} s^{-1}). The maximum fluorescence yield F_m in the dark-adapted state and F_m' in the light-adapted state were measured by applying a 0.7 s pulse of white light (PPFD of 3500 μmol m^{-2} s^{-1}, light source: KL 1500 electronic, Schott, Mainz, Germany). For the quenching analysis actinic white light (PPFD of 100 μmol m^{-2} s^{-1}, KL 1500 electronic) was provided. F_o' was measured with turning off the actinic light and applying 3 s of weak far-red light (102-FR, Walz, emission peak at 730 nm).

Thermoluminescence

Thermoluminescence measurements were carried out in a room with green safe light using a computerized setup described elsewhere (Zeinalov and Maslenkova 1996). Leaves segments were placed on a sample holder and covered with thin plastic plate. Thermoluminescence was excited by a continuous white light (PPFD of 240 μmol m^{-2} s^{-1}) during cooling the leaf discs from room temperature to −40°C or by single saturating (4 J) turnover flashes (10 μs half band with 1 Hz frequency) given at 5°C. After the flash exposure the samples were quickly cooled in liquid nitrogen. In the presence of PSII electron transport inhibitor DCMU flash excitation was at −10°C. The glow curves were recorded during warming to 60°C at a rate of 0.6°C/s.

Fluorescence imaging

For the fluorescence imaging at 440, 520, 690 and 740 nm compact flash-lamp fluorescence imaging system was used (Lichtenthaler and Babani 2000). The light of the exciting flashing Xenon lamp (16.7 Hz) was filtered by DUG 11 (Schott, Germany) filter to ensure the appropriate exciting wavelength (λ_{exc} = 360–370 nm) The detection of fluorescence images at the four wavelengths was performed via a CCD video camera using appropriate interference filters. Four hundred image accumulations were chosen as a suitable number of successive readout images. The images were corrected by the filter sensitivity parameters, by the background and by the inhomogeneity of the exciting light. The corrections and the arithmetical procedures were performed by software Camille 1.05 (Photonetics, Kehl, Germany).

Isolation of chlorophyll–protein complexes

Chloroplasts were isolated by the method of Mills and Joy (1980). Isolation of thylakoid membranes as well as the separation and identification of Chl–protein complexes was performed as described previously (Sárvári and Nyitrai 1994), shortly as it follows. Washed thylakoids were solubilised with mainly glucosidic detergents (dodecanoyl sucrose:nonyl glucoside:lithium dodecyl sulfate, 4.5:4.5:1, by weight), purchased from Sigma and Calbiochem–Novabiochem using a detergent to

Chl ratio of 10 (w/w). Chlorophyll–proteins were separated first by native gel electrophoresis ("green gels"). The thin slices of green gels were transferred to the top of a denaturating gels for a second dimension run according to the protocol of Laemmli (1970), but using 10–18% gel gradient. The relative amounts of Chl–protein bands separated by electrophoresis were determined as the percentage of Chl applied on the gel by densitometry of the scanned gels using the Phoretix software. The amounts of bands identified by their polypeptide pattern as belonging to a given complex were summed up. The absolute amounts of specific complexes were calculated in μg Chl g^{-1}DW by dividing the Chl content of 1 g dry leaf material among the complexes according to their percentage proportions.

Statistics

Control and water stress treatments were statistically compared. Comparison of means from two separate experiments, each in six replications was done by Student's *t* test.

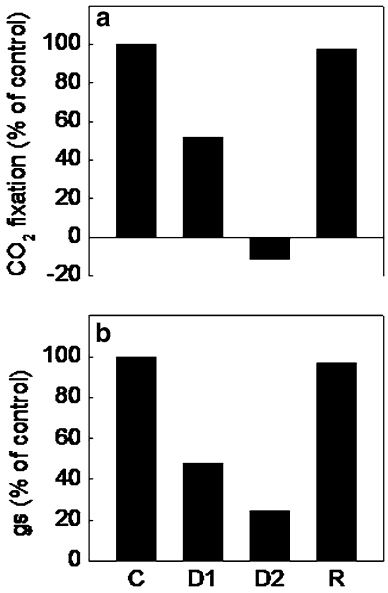

Fig. 1 Changes in net CO_2 assimilation (**a**) and stomatal conductance (**b**) rates in leaves of *H. rhodopensis* during moderate dehydration (stage D1, WC 1.51 g H_2O g^{-1}DW), desiccation (stage D2, WC 0.16 g H_2O g^{-1}DW) and after rehydration (R, WC 3.49 g H_2O g^{-1}DW). Measurements were carried out at 700 μmol photons m^{-2} s^{-1} photosynthetic active radiation. Note (−) indicates CO_2 release

Results

Haberlea plants were dehydrated with naturally occurring thin soil layers causing fast water loss. Leaf water content was reduced by 50% after 2 days and it was only 5% of the control after 7 days dehydration.

The pigment content showed no significant changes during dehydration (Table 1). It is not surprising, as *H. rhodopensis* is a homoiochlorophyllous desiccation-tolerant plant. Moreover, the desiccation was carried out at low light intensity (30 μmol m^{-2} s^{-1}), corresponding to the prevailing growth light intensity in natural conditions.

During the first phase of the desiccation (stage D1, 50% leaf WC) the rate of net CO_2 assimilation in the desiccating *H. rhodopensis* leaves declined by 50% and after 7 days of desiccation (stage D2, 5% leaf WC) there was no any net CO_2 assimilation rate, but the illuminated desiccated leaves have shown respiration activity (Fig. 1). Stomatal conductance (gs) decreased similarly to CO_2 assimilation rate over the first phase of desiccation and then declined slowly until the leaves dried out after 7 days, when gs value was 25% of the initial and control one (Fig. 1). The 0.81 value of Ci/Ca ratio (fully hydrated stage C) during the desiccation was increased up to 0.93, indicating a slight rate of CO_2 assimilation. Seven days after the start of rehydration (R) net CO_2 assimilation and stomatal conductance rates of the rehydrated and revived *H. rhodopensis* leaves were similar to the control ones (Fig. 1).

Maximal quantum efficiency of photosystem II (PSII) (F_v/F_m) decreased by 6 and 96% when the leaf water content was reduced by 50 and 95%, respectively (Fig. 2a). The reason for the low F_v/F_m values of dried leaves was that F_0 decreased to half of that of controls, while variable fluorescence F_v was almost completely abolished. Lack of variable fluorescence in light adapted state F_v' prevented the determination of

Table 1 Chlorophyll *a*, chlorophyll *b* and carotenoid content of control *Haberlea* leaves (C, WC 3.37 g H_2O g^{-1}DW) and after moderate dehydration (stage D1, WC 1.51 g H_2O g^{-1}DW), desiccation (stage D2, WC 0.16 g H_2O g^{-1}DW) and rehydration (R, WC 3.49 g H_2O g^{-1}DW)

Variant	Chl *a* (mg g^{-1} dry weight)	Chl *b* (mg g^{-1} dry weight)	Car (x + c) (mg g^{-1} dry weight)
C	2.67 ± 0.12	0.911 ± 0.030	1.050 ± 0.050
D1	3.01 ± 0.14	1.041 ± 0.050	1.020 ± 0.040
D2	2.46 ± 0.06	0.894 ± 0.015	0.985 ± 0.008
R	2.37 ± 0.20	0.882 ± 0.004	0.978 ± 0.009

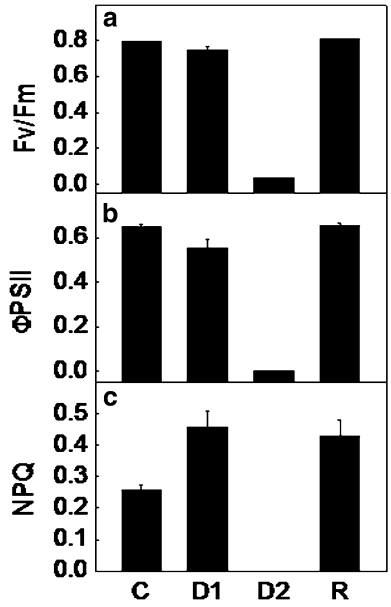

Fig. 2 Effect of moderate dehydration (stage D1, WC 1.51 g H_2O g^{-1}DW), desiccation (stage D2, WC 0.16 g H_2O g^{-1} DW) and rehydration (R, WC 3.49 g H_2O g^{-1}DW) of *H. rhodopensis* on the maximum quantum efficiency of PSII (*Fv/Fm*, **a**), the actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII, **b**) and non-photochemical chlorophyll fluorescence quenching (NPQ, **c**)

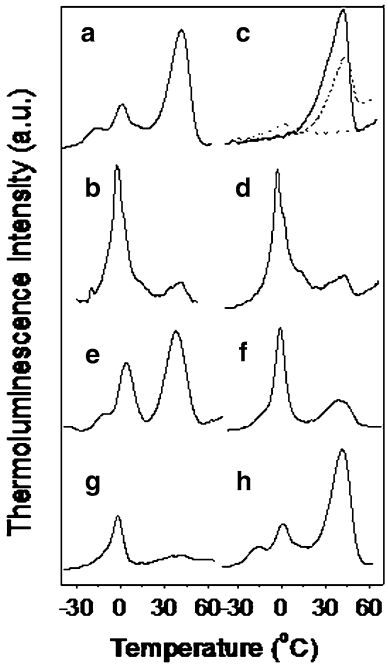

Fig. 3 Glow curve patterns from *Haberlea* leaves following dehydration and rehydration. Fully hydrated leaves after continuous illumination from room temperature to −40°C in the absence (**a**) or in the presence of 20 μM DCMU (**b**). B-band from control leaves illuminated with one single saturating flash given at 5°C (**c** *dashed line*) and after dehydration to 50% WC (**c** *dotted line*). Q-band from control leaves after flash illumination at −10°C (**d**). Thermoluminescence signals from control (**e**), 50% WC (**f**), 5% WC (**g**) and rehydrated leaves (**h**), illuminated with continuous white light

fluorescence induction parameters other than F_v/F_m in the desiccated state. Rewatering of the plants was followed by the recovery of water content, which was paralleled with recovery of both F_0 and F_m. The actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) was also dependent of the water status of the leaf (Fig. 2b). During desiccation both factors-fraction of open reaction centers, estimated by the photochemical quenching and the efficiency of excitation capture by open PSII reaction centers (F_v'/F_m') determining ΦPSII decreased similarly (data not shown). Upon rehydration ΦPSII increased back to the control value.

The thermal energy dissipation, expressed as non-photochemical quenching (NPQ) increased about 80% when the water content of *Haberlea* leaves was reduced from 3.37 to 1.51 g H_2O g^{-1}DW and it was still higher than the control after 1 week of rehydration (Fig. 2c).

The effect of desiccation and subsequent rehydration on the redox functioning of PSII donor and acceptor side components of *Haberlea* leaves was assessed by the changes in the main thermoluminescence bands during continuous and flash excitation. Representative glow curves from the leaves of fully hydrated *Haberlea* plants are shown in Fig. 3a–d. Thermoluminescence was excited with continuous white light during cooling the leaf disks from room temperature to −40°C (Fig. 3a). Under these experimental conditions a complex glow curve was obtained with three well-resolved thermoluminescence peaks at about −17, 0 and 45°C, which correspond to the so-called A, Q and B bands (Vass and Govindjee 1996). A and B-bands are though to arise from recombination of $S_3(His^+)Q_A^-$, and $S_{2,3}Q_B^-$, respectively and are very sensitive to DCMU, an inhibitor of the electron transfer between the primary, Q_A and the secondary, Q_B quinine acceptors. Q-band, corresponding to $S_2Q_A^-$ recombination was enhanced in the presence of DCMU (Fig. 3b). Illumination of dark adapted *Haberlea* leaves with a single flash (Fig. 3c), generating an $S_2Q_B^-$ charge pair recombination induced a thermoluminescence B-band at around 45°C. The amplitude of B-peak depends of the stage of hydration of the leaves. Infiltration of the leaves with DCMU leads to an incomplete suppression of B-band (Fig. 3d) and the appearance of one new peak at around 0°C ($S_2Q_A^-$).

The traces in Fig. 3e–f reveal that increasing dehydration resulted in changes in the overall intensity of thermoluminescence signals and redistribution of the

thermoluminescence emission between the existing Q and B peaks with practically unchanged peak temperatures. In desiccated leaves, the amplitude of the thermoluminescence B-band sharply decreased and mainly a charge recombination related to $S_2Q_A^-$ takes place (Fig. 3g). After rehydration the thermoluminescence glow curve pattern resembles that of the control leaves with the relative contribution of B-band being maximal (Fig. 5h).

A comparative study of thermoluminescence oscillation pattern related to the water content of the leaves is shown in Fig. 4. A small shoulder at 0°C, which corresponds to $S_2Q_A^-$ (Q-band) was occasionally observed in control leaves (Fig. 4a), became well expressed in severely dehydrated *Haberlea* leaves (Fig. 4b). The amplitudes of both Q and B peaks oscillate in a complementary way. Rehydration completely restored the shape and the amplitudes of the main thermoluminescence B-peak after flash illumination (Fig. 4c).

The fluorescence imaging system offers the possibility to show the variation of the fluorescence signal over the whole leaf area in blue, green, red and far-red spectral regions. The fluorescence ratios blue/red and blue/far-red proved to be very good stress indicators (Buschmann et al. 2000). We found that these ratios increased 2.5 times after 50% reduction of water content and 8–9 times when water content was reduced more than 90% (Fig. 5). It was due to a stronger increase in the blue and green fluorescence and a decrease in red and far-red fluorescence.

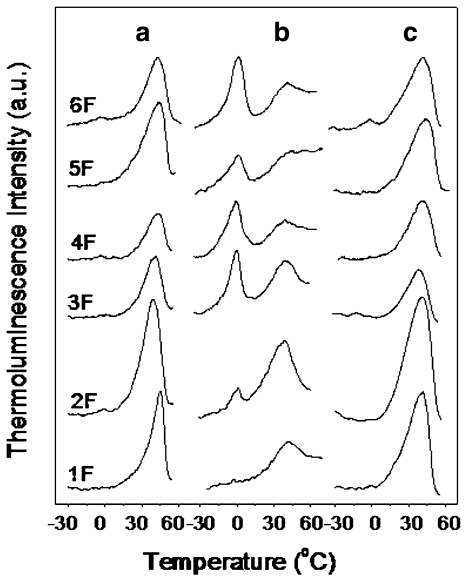

Fig. 4 Thermoluminescence B-band emission from fully hydrated (**a**), severely dehydrated (**b**) and rehydrated (**c**) *Haberlea* leaves as a function of the number of the flashes (*F*), from 1 to 6, given at 5°C

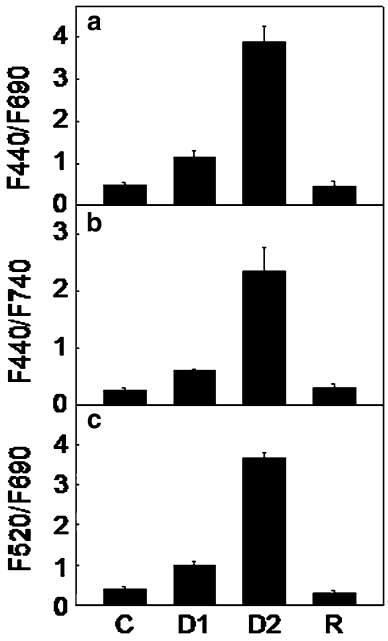

Fig. 5 Changes in the fluorescence ratios blue/red (**a**), blue/far-red (**b**) and green/red (**c**) during moderate dehydration (stage D1, WC 1.51 g H_2O g^{-1}DW), desiccation (stage D2, WC 0.16 g H_2O g^{-1}DW) and after rehydration (R, WC 3.49 g H_2O g^{-1}DW) of *H. rhodopensis*

Chlorophyll–protein composition of thylakoids was determined by native PAGE (Fig. 6a). Twelve green bands were separated from the thylakoids, which had analogous polypeptide patterns to those of other higher plants (Sárvári and Nyitrai 1994). The corresponding bands of samples being in different water stage were of similar composition. Bands 1, 2, 3, and 4 contained PSI particles differing in antenna size and content, from which the largest one also bound LHCII in addition to LHCI. Most LHCII was present in trimeric state in band 8. The components of PSII core complex and its connecting antenna were present in bands 5–8 and 9–11 as partially solubilised complexes and monomeric components, respectively. Some PSII, probably dimeric, run together with PSI in band 2, the amount of which was estimated by comparing the amount of CP47 apoproteins present in the PSI bands and in a pure PSII band, respectively. The fastest migrating band contained free pigments (largely carotenoids) representing about 10% of the total scanned pigments on the gel.

Concomitant with the mostly unchanged Chl *a/b* ratios of leaves and isolated thylakoids, there were only slight, not significant differences in the relative amounts of Chl-proteins among the control, desiccated and recovered samples (Fig. 6a) similarly to their mostly invariable thylakoid polypeptide patterns

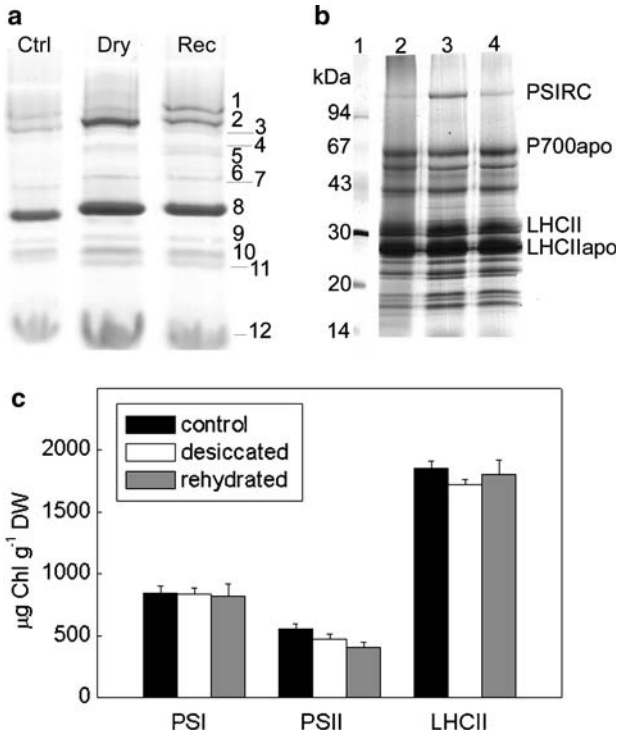

Fig. 6 Chlorophyll–protein patterns of thylakoids (**a**), and polypeptide patterns of thylakoids (**b**). *Lane 1* standard proteins, Ctrl; *lane 2* control, Dry; *lane 3* desiccated samples, Rec; *lane 4* recovered samples. **b** *apo* apoprotein; *RC* reaction centre (containing the two large proteins of PSI). Thylakoids were denatured at room temperature for 1 h. Protein standards (kDa): phosphorylase b (94), bovine serum albumin (67), ovalbumin (43), carbonic anhydrase (30), soybean tripsin inhibitor (20), α-lactalbumin (14); **c** Absolute amounts of chlorophyll–proteins in thylakoids, isolated from control, desiccated and rehydrated *H. rhodopensis* plants

(Fig. 6b). The calculated densitograms, however, tendentiously showed a slight relative increase in the amount of PSI/P700 apoprotein and decrease in LHCII/apo-LHCII in the dried samples (not shown). The most prominent change was observed in the ratio of band 1–band 2, which decreased from 1.09 ± 0.23 in the control to 0.28 ± 0.08 in desiccated samples, i.e. the LHCII content of PSI also decreased due to dehydration, and it was restored following the rehydration period (1.12 ± 0.09). A parallel increase in the P700 reaction centre band was observed in the denatured polypeptide pattern of the dry sample. In an absolute scale, the small decrease in the Chl content observed in desiccated and recovered leaves compared to the control was discovered to be due to the somewhat decreased amount of LHCII in desiccated samples, and to the slightly lowered amount of PSII core in the recovered samples, respectively (Fig. 6c).

The 77 K fluorescence emission spectra reflected a general fluorescence decrease in the desiccated sample, which was stronger in the PSII than the PSI region of the spectrum (data not shown). Fluorescence (77 K) excitation spectra of the 740 nm emission showed a minute decline in the Chl *b* (650 nm) and in the shorter wavelength Chl *a* component in accordance with the LHCII decline in PSI (data not shown). The changes observed in dried tissues mostly recovered following rehydration.

Discussion

During the first phase of the desiccation the net CO_2 assimilation decline in desiccating *H. rhodopensis* leaves was influenced by stomatal closure. However, the further lowering and an eventual stop of net CO_2 assimilation was caused by both the decrease in stomatal conductance and in the photochemical activity (Figs. 1, 2), similarly to other vascular homoiochlorophyllous desiccation-tolerant plants (Schwab et al. 1989).

Stromal enzymes are able to fix CO_2 at −38 and −22 MPa osmotic potentials (Dilks and Proctor 1979; Nash et al. 1990), even in the desiccation intolerant mesophytes (Kaiser et al. 1981). Enzymes involved in respiration also retain their activity at low water potentials (Bewley 1979; Dilks and Proctor 1979; Nash et al. 1990). Respiration was also detected at extremely low water content in desiccating poikilochlorophyllous desiccation-tolerant *X. scabrida* leaves (Tuba et al. 1996). At this level of hydration (stage D2) the water is still bound to macromolecules although it has some properties of bulk water such as allowing diffusion of solutes and hydrophobic interactions between macromolecules (Smirnoff 1993). This indicates that in desiccation-tolerant plants during desiccation the stromal and respiratory enzymes are only inactivated (but not degraded) and the inactivation is completed only in the final desiccated stage where all liquid water was lost. This also explains the measured CO_2 incorporation and respiration activity of *H. rhodopensis* leaves in the prolonged desiccated stage. All of these suggestions are supported by the not fully closed stomata and the 0.93 Ci/Ca ratio in the well developed, completed stage of desiccation (D2). After rehydration of leaves was regained the CO_2 assimilation was fully restored (Fig. 1).

Our results also showed that 50% reduction in water content of *Haberlea* leaves induced smaller decrease in the quantum yield of PSII photochemistry than in CO_2 fixation (Figs. 1, 2). This suggests that photosynthetic electrons were used for other photochemical processes under the stomatal limitation of photosynthesis. Both increased photorespiration and increased allocation of

electrons to oxygen under water stress have been suggested as possible mechanisms by which excess photochemical energy can be dissipated and high quantum yield of PSII electron transport can be maintained, thus protecting PSII from damage (Lu and Zhang 1998). Like other homoiochlorophyllous desiccation-tolerant plants (Proctor and Tuba 2002; Farrant et al. 2003) the potential danger of photoinhibition and the appearance of reactive oxygen species in dehydrated *Haberlea* leaves is considerable. In nature, *H. rhodopensis* usually occupies shaded areas. Another adaptive feature is an extensive shrinkage and folding of the leaves to avoid light-induced damages. The increased non-radiative energy dissipation at middle stage of water loss also contributed for protecting PSII from photoinhibition (Fig. 2c).

Detailed information about the ability of PSII reaction centers to perform photochemical charge separation during desiccation of *Haberlea* plants was obtained by thermoluminescence measurements. Data from inhibitor experiments (Fig. 3b, d) and the observed oscillations in flash experiments (Fig. 4) show that the peaks with thermoluminescence maximum (T_m) at 0 and 45°C could be assigned to Q ($S_2Q_A^-$) and B ($S_2Q_B^-$) bands. Changes in the contribution of these two main thermoluminescence bands to the total thermoluminescence emission started at the beginning of dehydration (Fig. 3e) and below 50% water content charge recombination related to $S_2Q_A^-$ (Q-band) strongly increased in parallel to the decrease of B-band ($S_2Q_B^-$). Subsequent rehydration of *Haberlea* leaves completely restore the shape and the intensity of thermoluminescence glow curves that means electron transport between the primary and secondary quinine acceptors was reversibly modified. We suggested that the increased contribution of $S_2Q_A^-$ charge recombination in dehydrated *Haberlea* leaves served to protect Q_B site from over excitation. Scotnica et al. (2000) reported the appearance of Q-band in thermoluminescence glow curves of desiccating barley leaves as a consequence of inhibition of electron transport between primary and secondary quinone acceptors and suggest an increase in Q_B-non-reducing PSII centers. Reversible changes in the amplitude and oscillation pattern of B- and Q-band, obtained during flash illumination (Fig. 4) suppose that some changes in the kinetic characteristics of S_2 and S_3 states of PSII donor side during desiccation cannot be excluded. It was demonstrated (Peeva and Maslenkova 2004) that $S_2Q_B^-$ state of PSII in homoiochlorophyllous desiccation-tolerant *Haberlea* was stabilized, evidenced by an unusually high T_m of B-peak (45–47°C) and by an increase in the lifetime of S_2 state. In addition a part of these centers was less susceptible to DCMU (Peeva and Maslenkova 2004). 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 the charge recombination in the rest operating centers were considered to indicate modifications of the redox properties of Q_B (Georgieva et al. 2005) related to desiccation tolerance of *Haberlea*. It is reasonably to suggest these modifications to favor $S_2Q_A^-$ charge recombination under desiccation. The increased population of Q_A^- enhances the probability for non-radiative energy dissipation and represents an effective mechanism of protection.

In spite of the cessation of CO_2 fixation and the strong decrease of PSII function in dehydrated *Haberlea*, the integrity of thylakoids was preserved and the amount and ratio of pigment–protein complexes hardly changed (Fig. 6). A non-significant decrease in the LHCII amount was observed and accompanied with a slight drop of the LHCII content of PSI. The trend of decreasing the amount of PSII core in the dehydrated samples (15%) continued even during rehydration (up to 25%), which could be attributed to damage of some PSII centers. However, the remaining PSII centers were fully active and able to assure the energy supply, which could be considered as a protective mechanism in *Haberlea* for maintaining photosynthesis near to control levels. High stability of the thylakoid pigment–protein complexes was reported also for the homoiochlorophyllous desiccation-tolerant plant *Boea hygrometrica* (Deng et al. 2003), suggesting that preserving the integrity of pigment–protein complexes of photosystems is essential for these plants to survive under extreme drought conditions.

During dehydration of *Haberlea* leaves the blue and green fluorescence emission increased and it was extremely high in desiccated leaves (Fig. 5). The blue and green fluorescence has been generally attributed to cell wall bound ferulic acid, although the contribution of flavonoids and other simple phenols, such as coumaric acid, has been also suggested (Buschmann et al. 2000; Apostol et al. 2003). The contribution of flavonoid compounds to the blue and green fluorescence was confirmed using the mutant deficient in flavonoids. Even the smaller increase in blue and green fluorescence in drought stressed barley as compared to untreated material, suggested increased polyphenolic synthesis, since drought is also known to stimulate polyphenolics (Hideg et al. 2002).

In summary, our results showed that during desiccation of whole *Haberlea* plants under conditions similar to natural conditions the damage of the photosynthetic apparatus was limited to a repairable level and the

physiological integrity was maintained in the dry state enabling full recovery after rehydration. The lack of changes in both chlorophyll content and amounts of chlorophyll–proteins, together with the reversible modifications in PSII electron transport, some peculiarities in charge separation and stabilization of PSII as well as increased polyphenolic synthesis during desiccation could contribute to drought resistance and fast recovery after rehydration of *Haberlea* plants.

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References

Alamillo JM, Bartels D (2001) Effects of desiccation on photosynthesis pigments and the ELIP-like dsp 22 protein complex in the resurrection plant *Craterostigma plantagineum*. Plant Sci 160:1161–1170

Alisson SD, Chang B, Randolph TW, Carpenter JF (1999) Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. Arch Biochem Biophys 365:289–298

Apostol S, Viau AA, Tremblay N, Briantais J-M, Prasher S, Parent L-E, Moya I (2003) Laser-induced fluorescence signatures as a tool for remote monitoring of water and nitrogen stresses in plants. Can J Remote Sens 29:57–65

Bewley JD (1979) Physiological aspects of desiccation tolerance. Annu Rev Plant Physiol 30:195–238

Buschmann C, Langsdorf G, Lichtenthaler HK (2000) Imaging of blue, green, and red fluorescence emission of plants: an overview. Photosynthetica 38:483–491

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387

Crowe JH, Hoekstra FA, Nguyen KHN, Crowe LM (1996) Is vitrification involved in depression of the phase transition temperature in dry phospholipids. Biochim Biophys Acta 1280:187–196

Deng X, Hu Z-A, Wang H-X, Wen X-G, Kuang T-Y (2003) A comparison of photosynthetic apparatus of the detached leaves of the resurrection plant *Boea hygrometrica* with its non-tolerant relative *Chirita heterotrichia* in response to dehydration and rehydration. Plant Sci 165:851–861

Dilks TJK, Proctor MCF (1979) Photosynthesis, respiration and water content in bryophytes. New Phytol 82:97–114

Farrant JM, Sherwin HS (1998) Mechanisms of desiccation tolerance in seeds and resurrection plants. In: Taylor AG, Huang X-L (eds) Progress in seed research. Proceedings of 2nd International Conference on Seed Science and Technology, Geneva, NY, Communication Services of New York State Agricultural Experiment Station, pp 109–120

Farrant JM, Cooper K, Kruger LA, Sherwin HW (1999) The effect of drying rate on the survival of three desiccation-tolerant angiosperm species. Ann Bot 84:371–379

Farrant JM, Willigen CW, Loffell DA, Bartsch S, Whittaker A (2003) An investigation into the role of light during desiccation of three angiosperm resurrection plants. Plant Cell Environ 26:1275–1286

Georgieva K, Maslenkova L, Peeva V, Markovska Yu, Stefanov D, Tuba Z (2005) Comparative study on the changes in photosynthetic activity of the homoiochlorophyllous desiccation-tolerant *Haberlea rhodopensis* and spinach leaves during desiccation and rehydration. Photosynth Res 85:191–203

Hideg E, Juhasz M, Bornman JF, Asada K (2002) The distribution and possible origin of blue-green fluorescence in control and stressed barley leaves. Photochem Photobiol Sci 1:934–941

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431–438

Kaiser WM, Kaiser G, Schöner S, Neimanis S (1981) Photosynthesis under osmotic stress. Differential recovery of photosynthetic activities of stroma enzymes, intact chloroplast and leaf slices after exposure to high solute concentrations. Planta 153:430–435

Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685

Lichtenthaler KH (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382

Lichtenthaler HK, Babani F (2000) Detection of photosynthetic activity and water stress by imaging the red chlorophyll fluorescence. Plant Physiol Biochem 38:889–895

Lu C, Zhang J (1998) Effects of water stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants. Aust J Plant Physiol 25:883–892

Mills WR, Joy KW (1980) A rapid method for isolation of purified, physiologically active chloroplasts, used to study the intracellular distribution of amino acids in pea leaves. Planta 148:75–83

Nash III TH, Reiner A, Demmig-Adams B, Kilian E, Kaiser WM, Lange OL (1990) The effect of atmospheric desiccation and osmotic water stress on photosynthesis and dark respiration of lichens. New Phytol 116:269–276

Peeva V, Maslenkova L (2004) Thermoluminescence study of photosystem II activity in *Haberlea rhodopensis* and spinach leaves during desiccation. Plant Biol 6:1–6

Proctor MCF, Tuba Z (2002) Poikilohydry and homoihydry: antithesis or spectrum of possibilities? New Phytol 156:327–349

Sárvári É, Nyitrai P (1994) Separation of chlorophyll–protein complexes by Deriphat polyacrylamide gradient gel electrophoresis. Electrophoresis 15:1067–1071

Sass L, Csintalan Zs, Tuba Z, Vass I (1996) Thermoluminescence studies on the function of photosystem II in the desiccation tolerant lichen *Cladonia convoluta*. Photosynth Res 48:205–212

Schwab KB, Schreiber U, Heber U (1989) Response of photosynthesis and respiration of resurrection plants to desiccation and rehydration. Planta 177:217–227

Scotnica J, Matouskova M, Naus J, Lazar D, Dvorak L (2000) Thermoluminescense and fluorescence study of changes in photosystem II photochemistry in desiccating barley leaves. Photosynth Res 65:29–40

Scott P (2000) Resurrection plants and the secrets of eternal leaf. Ann Bot 85:159–166

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Tuba Z, Lichtenthaler HK, Csintalan Zs, Nagy Z, Szente K (1994) Reconstitution of chlorophylls and photosynthetic CO_2 assimilation upon rehydration in the desiccated poikilochlorophyllous plant *Xerophyta scabrida*. Planta 192:414–420

Tuba Z, Csintalan Zs, Proctor MCF (1996) Photosynthetic responses of a moss, *Tortula ruralis* (Hedw.) Gaertn. et al. ssp.

ruralis, and the lichens *Cladonia convoluta* (Lam.) P. Cout. and *C. furcata* (Huds.) Schrad. to water deficit and short periods of desiccation, and their eco-physiological significance: a baseline study at present-day CO_2 concentration. New Phytol 133:353–361

Tuba Z, Proctor MCF, Csintalan Zs (1998) Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: a comparison and an ecological perspective. Plant Growth Regul 24:211–217

Vass I, Govindjee (1996) Thermoluminescence from the photosynthetic apparatus. Photosynth Res 48:117–126

Vecchia FD, Asmar TE, Calamassi R, Rascio N, Vazzana C (1998) Morphological and ultrastructural aspects of dehydration and rehydration in leaves of *Sporobolus stapfianus*. Plant Growth Regul 24:219–228

Zeinalov Yu, Maslenkova L (1996) A computerised equipment for thermoluminescence investigations. Bulg J Plant Physiol 22:88–94