PHOTOSYNTHETICA 46 (2): 208-215, 2008

# Responses of the resurrection plant *Haberlea rhodopensis* to high irradiance

K. GEORGIEVA[*,+], S. LENK[**], and C. BUSCHMANN[**]

*Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, BG-1113 Sofia, Bulgaria[*]*
*Botanisches Institut II (Molekularbiologie und Biochemie der Pflanzen), Universität Karlsruhe, Kaiserstrasse 12, D-76128 Karlsruhe, Germany[**]*

## Abstract

The effect of high irradiance (HI) during desiccation and subsequent rehydration of the homoiochlorophyllous desiccation-tolerant shade plant *Haberlea rhodopensis* was investigated. Plants were irradiated with a high quantum fluence rate (HI; 350 μmol $m^{-2}$ $s^{-1}$ compared to *ca*. 30 μmol $m^{-2}$ $s^{-1}$ at the natural rock habitat below trees) and subjected either to fast desiccation (tufts dehydrated with naturally occurring thin soil layers) or slow desiccation (tufts planted in pots in peat-soil dehydrated by withholding irrigation). Leaf water content was 5 % of the control after 4 d of fast and 19 d of slow desiccation. *Haberlea* was very sensitive to HI under all conditions. After 19 d at HI, even in well-watered plants there was a strong reduction of rates of net photosynthesis and transpiration, contents of chlorophyll (Chl) and carotenoids, as well as photosystem 2 activity (detected by the Chl fluorescence ratio $R_{Fd}$). Simultaneously, the blue/red and green/red fluorescence ratios increased considerably suggesting increased synthesis of polyphenolic compounds. Desiccation of plants in HI induced irreversible changes in the photosynthetic apparatus and leaves did not recover after rehydration regardless of fast or slow desiccation. Only young leaves survived desiccation.

*Additional key words*: carotenoids; chlorophyll fluorescence; desiccation tolerant plant; drought stress; fluorescence imaging; leaf area; net photosynthetic rate; transpiration rate; water use efficiency.

## Introduction

The poikilohydric or resurrection plants posses the unique ability to withstand desiccation of their vegetative tissues and to revive from an air-dried state (Gaff 1989). These plants provide very suitable model for the study of mechanisms underlying desiccation tolerance. Desiccation-tolerant plants are usually subdivided into homoiochlorophyllous plants retaining their chlorophyll (Chl) during desiccation and poikilochlorophyllous plants where desiccation results in the loss of Chl which must be re-synthesized following rehydration (Tuba *et al*. 1998). The European flowering homoiochlorophyllous desiccation-tolerant plant species are restricted to two genera (*Ramonda* and *Haberlea*) of the family Gesneriaceae. *Haberlea rhodopensis* Friv. is a tertiary paleophytic relict on the Balkan Peninsula. Recently, it inhabits sun exposed or shaded, northern, chiefly limestone slopes in mountain zones with relatively high humidity. It is a perennial herbaceous rock resurrection plant, forming dense tufts of leaves, every rosette bearing in spring one to five flower-stalks.

Our previous investigations have shown that detached *Haberlea* leaves as well as whole plants were able to survive desiccation in the dark or at low irradiance (about 30 μmol $m^{-2}$ $s^{-1}$) to water content below 10 % with photosynthetic activity fully recovered after rehydration (Georgieva *et al*. 2005, 2007). Under mild drought (water content up to 50 %) the decline of net photosynthetic rate ($P_N$) was influenced largely by stomata closure (Georgieva *et al*. 2007). Further lowering of $P_N$ was caused by both the decrease in stomatal conductance and in photo-

*Received* 12 September 2007, *accepted* 1 November 2007.
[+]Corresponding author; fax: +359 2 873 99 52, e-mail: katya@bio21.bas.bg
*Abbreviations:* Chl – chlorophyll; CWL – central wavelength; DM – dry mass; FD – fast desiccation; $^{B}$F690 – fluorescence at 690 nm excited with blue radiation; $^{UV}$F440, $^{UV}$F550, $^{UV}$F690 – fluorescence at 440, 550 and 690 nm, respectively, excited with ultraviolet radiation; FWHM – full width-half maximum; HI – high irradiance; PAR – photosynthetically active radiation; $P_N$ – net photosynthetic rate, PS2 – photosystem 2; $R_{Fd}$690 – ratio of fluorescence decrease determined at 690 nm; SD – slow desiccation; UV – ultraviolet; WUE – water use efficiency.
*Acknowledgement*: This study was carried out during a stay of K.G. at the University of Karlsruhe financed by the Deutsche Forschungsgemeinschaft (DFG). S.L. has been research fellow of the Research Training Network STRESSIMAGING financed by the European Community in the Human Potential Programme (contract HPRN-CT-2002-00254). We acknowledge the reviewer's comments.

chemical activity of photosystem 2 (PS2). Unchanged Chl content and the maintenance of Chl-protein-complexes, reversible modifications in PS2 electron transport, and enhanced probability for non-radiative energy dissipation during desiccation of *Haberlea* contribute to drought resistance and fast recovery after rehydration (Georgieva *et al*. 2007).

The irradiation during desiccation can extremely damage photosynthetically active tissues. Under water-limiting conditions, photon energy taken up by Chl can not be used for photosynthesis and can lead to the formation of oxygen free radicals, which may cause considerable sub-cellular damage (Powles 1984, Smirnoff 1993, Farrant *et al*. 2003).

The angiosperm resurrection plants have a number of mechanisms to minimize photo-oxidative damage including up-regulation of antioxidant system (Sherwin and Farrant 1998). In addition to photochemical protection, resurrection plants undergo anatomical changes to prevent irradiance-Chl interaction and thereby put a stasis on photosynthesis and photo-oxidation reactions (Farrant *et al*. 2003). *Craterostigma wilmsii* and *Myrothamnus flabellifilius* use leaf folding and anthocyanin accumulation as a means minimizing photo-oxidative damage (Sherwin and Farrant 1998). Studies on the resurrection fern *Polypodium polypoides* have shown more severe damage with longer recovery times, when plants were dried under high irradiance (HI) compared to low irradiance (Muslin and Homann 1992). Data on the effect of radiant energy on photosynthetically active tissues during desiccation of homoiochlorophyllous resurrection plants are scarce and restricted to *C. wilmsii* and *M. flabellifilius*.

The aim of the present study was to examine the effect of HI (350 μmol $m^{-2}$ $s^{-1}$, more than 10 times higher than at the natural habitat) on the homoiochlorophyllous desiccation-tolerant plant *H. rhodopensis* underlying desiccation and subsequent rehydration. The leaves were characterized by their water and pigment content, $P_N$ and transpiration rate ($E$), as well as their PS2 fluorescence induction (vitality index fluorescence decrease ratio $R_{Fd}$, Lichtenthaler and Rinderle 1988) and multicolour fluorescence images.

## Materials and methods

**Plants**: Well-hydrated *H. rhodopensis* plants were collected from their natural habitat (the vicinity of Asenovgrad, Bulgaria) where they grow on rocks below trees under irradiance of less than 30 μmol $m^{-2}$ $s^{-1}$. Adult rosettes from the same locality and of similar size and appearance were selected for the experiments. The tufts were transferred in a chamber at 28/20 °C day/night temperature, irradiance of 350 μmol $m^{-2}$ $s^{-1}$, 12/12 h day/night cycle, and relative humidity of 60 %. Plants were subjected to drought stress by two different protocols: (*1*) tufts dehydrated with naturally occurring thin soil layers (fast desiccation, FD), and (*2*) tufts planted in pots in peat-soil and dehydrated by withholding irrigation (slow desiccation, SD). After desiccation, the plants were rehydrated for 7 d by spraying water on the leaves to simulate rainfall and keeping the soil damp. Control plants were regularly watered throughout the experiment. The data are given for mature leaves unless it is stated that young leaves are being discussed.

**Water content, pigment analysis, and area of leaves**: Water content of the leaves was determined gravimetrically by weighing the leaves before and after oven drying at 80 °C to a constant mass. The leaf area was determined using an area meter (*LI-3000*, *LI-COR*, Lincoln, USA) with the flat leaves enclosed in a transparent foil. Leaf pigments were extracted from the leaves. Circular pieces of 10-mm in diameter were cut from the leaves by means of a cork puncher and extracted with 100 % acetone using a mortar. The extract was centrifuged for 5 min at 500×*g* in order to have a fully transparent extract without light scattering by fibres and coagulated proteins. The concentrations of Chl *a* and *b* as well as of total carotenoids were determined spectrophotometrically (*UV-2101PC*, *Shimadzu*, Düsseldorf, Germany) from the acetone extracts using the equations of Lichtenthaler (1987).

**$CO_2$ gas exchange and transpiration**: $P_N$ and $E$ were measured using a $CO_2$/$H_2O$-porometer (*Walz*, Effeltrich, Germany). The measurement was carried out for 5 min in the dark and during a subsequent irradiation period of 30 min (PAR: 800 μmol $m^{-2}$ $s^{-1}$). From the ratio $P_N/E$ the water use efficiency (WUE) was calculated.

**Ratio of fluorescence decrease** ($R_{Fd}$) was determined from the induction kinetics of the Chl fluorescence measured using a two-wavelength Chl fluorometer (*CFM 636973*, *Opto-Sens*, Budapest, Hungary). With this instrument Chl fluorescence is excited at 635 nm and measured at the two fluorescence bands 690 and 735 nm (for details see Barócsi *et al*. 2000). Circular pieces of 10 mm diameter were cut from the leaves by means of a cork puncher and dark-adapted for 20 min before the measurements. The $R_{Fd}$-values were calculated for the fluorescence at 690 nm from the maximum ($F_{max}$) about 500 ms after onset of excitation and the steady state fluorescence ($F_s$) 5 min after onset of excitation: $R_{Fd} = (F_{max} - F_s)/F_s$.

**Fluorescence images** were acquired using a multi-colour imaging system described by Lenk and Buschmann (2006). The fluorescence was excited by a Xenon flash lamp (*FX-4400*, *Perkin Elmer Optoelectronics*, Cambridge, UK) from a distance of 0.5 m (input energy

0.5 J per flash, repetition rate 50 Hz; manufactured by I. Péczeli and L. Kocsányi at the Budapest University of Technology and Economics). Four excitation filters were inserted in front of the Xenon lamp: (*a*) either a UV filter (*DUG 11*, *Schott*, Mainz, Germany) with a central wavelength (CWL) at 340 nm and a full with-half bandwidth (FWHM) of 75 nm or (*b*) a blue filter (*BG-12*, *Schott*, Mainz, Germany) with a CWL at 407 nm and a FWHM of 104 nm together with a UV cut-off filter (*Edmund Optics*, Karlsruhe, Germany) protecting the blue filter from UV damage. The two filters for blue excitation result in a CWL at 423 nm and a FWHM of 79 nm. Fluorescence images were taken using a gated intensified video camera (*Optronis*, Kehl, Germany) with a lens of 50 mm focal length. Inside the camera is an image intensifier tube with a micro channel plate (2nd generation, *S25* photocathode, *P43* screen, gate-able up to 50 ns, coupling by a fibre reducing taper). The intensifier is synchronized to the Xenon flashes with a gating time of 20 μs. The filter wheel located behind the lens inside the camera allows fluorescence measurements at different wavelength ranges. Three filters (*LOT-Oriel*, Darmstadt, Germany) were used: a blue (CWL = 440 nm, FWHL = 10 nm), a green (CWL = 550 nm, FWHL = 40 nm), and a red (CWL = 692 nm, FWHL = 10 nm) filter.

The images were transferred to a PC *via* a frame grabber (*Meteor II*, *Matrox*, Québec, Canada). For each sample 200 images were accumulated and the same number of images, taken subsequently without excitation radiation, was subtracted as background. Inhomogeneous distribution of the Xenon lamp was corrected by an image of the blue fluorescence emitted by white paper which exhibits intensive fluorescence under UV excitation. The image acquisition is controlled by a PC using special imaging software (*BHR-Camille 1.05*, *EHR*, Pforzheim, Germany) developed for this system. For image analysis freely available imaging software (*ImageJ*) was used (Rasband 1997–2008).

**Statistics**: The data for control and water stress treatments were statistically analyzed. Means from 6 replications (6 leaves from different plants) were compared by Student's *t*-test.

## Results

*Haberlea* plants growing under irradiance of about 30 μmol $m^{-2}$ $s^{-1}$ in natural habitat were subjected to either FD or SD at irradiance of 350 μmol $m^{-2}$ $s^{-1}$ (HI). Control plants were regularly watered and exposed to HI. Exposure of well-watered (control) *Haberlea* plants to HI gradually decreased the water content of leaves and it was 27 % lower than the initial one after 19 d (Fig. 1*A*). SD-plants of *Haberlea* took 19 d to reach an air-dried state, whereas in FD-plants the same water content was achieved in 4 d (Fig. 1*A*). Upon 7 d of rehydration the water content of fully expanded leaves increased only by approximately 10 % [from 0.10 to 0.24 kg($H_2O$) $kg^{-1}$(DM)] and 20 % [from 0.12 to 0.70 kg($H_2O$) $kg^{-1}$(DM)] for the FD and SD plants, respectively. The youngest leaves were also desiccated to air-dry state after FD or SD. However, after rehydration their water content was almost back to the initial one (Fig. 1*A*).

The leaf area of control *Haberlea* plants was reduced by 10 % (from 23.7 to 21.4 $cm^2$) after 19 d of growth at HI (Fig. 1*B*). However, an extensive shrinkage of the leaves occurred during desiccation leading to 60 % (from 22.7 to 9.2 $cm^2$) and 75 % (from 21.5 to 5.5 $cm^2$) reduction of leaf area of SD and FD plants, respectively. SD procedure in young leaves led to similar reduction in their leaf area (about 60 %, from 17.7 to 7.4 $cm^2$) as in fully developed leaves (Fig. 1*B*). The area of fully expanded leaves increased only by 5 % after rehydration (last column in Fig. 3*B*) whereas in the younger leaves it was fully recovered (Fig. 1*B*, last column in Fig. 3*C*).

Treatment of well-watered plants at HI gradually decreased the pigment content but it was stronger in FD-leaves (Fig. 1*C,D*). The contents of Chl *a* and *b* as well as of total carotenoids decreased by about 30 and 20 %, respectively, in both fully expanded and younger leaves in air-dried state. After rehydration of plants the pigment contents of fully developed leaves increased by 10 % whereas they were very close to the initial ones in young leaves (Fig. 1*C,D*).

The $P_N$ of control plants was reduced by about 50 % after 4 d at HI and it was only 5 % [0.148 μmol($CO_2$) $m^{-2}$ $s^{-1}$] of the initial one after 19 d (Fig. 2*A*). $P_N$ additionally decreased as a result of SD. When *H. rhodopensis* was rapidly desiccated, $P_N$ was inhibited by 90 % (from 3.026 to 0.346 μmol $m^{-2}$ $s^{-1}$) already after 2 d. The *E* declined significantly as a result of both irradiation and drying in the fully expanded leaves and it was less affected in the youngest leaves up to 11 d of desiccation (Fig. 2*B*). The exposure of *Haberlea* to HI reduced the water use efficiency (WUE, Fig. 2*C*), especially for the dehydrated plants. After rehydration, $P_N$ in fully expanded and youngest leaves was 0.084 μmol $m^{-2}$ $s^{-1}$ (about 3 % of the initial one) and 1.746 μmol $m^{-2}$ $s^{-1}$ (about 60 % of the initial one), respectively.

The $R_{Fd}690$ as a measure of photochemical activity and vitality of plants (Lichtenthaler and Rinderle 1988) was reduced by approximately 40 % after 19 d of HI treatment (Fig. 2*D*). The $R_{Fd}$ values decreased as a result of SD and were strongly inhibited when leaf water content was reduced by about 60 %. Similarly to the other investigated parameters, $R_{Fd}$-values significantly decreased after 2 d of FD (Fig. 2*D*). The value of $R_{Fd}690$ measured after rehydration of fully expanded leaves was 0.096, but it was 2.150 in the youngest leaves.

Fluorescence images showed during the desiccation

the shrinkage of the leaves and an increase in the blue and green fluorescence ($^{UV}$F440 and $^{UV}$F550) as well as a decrease in the Chl fluorescence detected at 690 nm and excited in the UV and in the blue radiation ($^{UV}$F690 and $^{B}$F690) (see Table 1 and Fig. 3*B*). The inverse changes in the blue and green as well as in the Chl fluorescence resulted in an increase of the ratio between the blue (or green) fluorescence to the Chl fluorescence (see ratios $^{UV}$F440/$^{UV}$F690 and $^{UV}$F550/$^{UV}$F690 in Table 1) during desiccation. The leaves did not recover from the FD. Only in few plants small parts of some leaves recovered which was also detectable as an increased Chl fluorescence after 14 d (data not shown). In case of SD, parts of leaves kept more often lower blue and green fluorescence and higher Chl fluorescence (Fig. 3*B*) than during FD. Only the young leaves showed full recovery (Fig. 3*C*).

## Discussion

*H. rhodopensis* is very sensitive to HI. Exposure of plants to 350 μmol $m^{-2}$ $s^{-1}$ for 19 d resulted in decreased pigment content, reduced PS2 activity, and inhibited $CO_2$ assimilation. Moreover, desiccation of *Haberlea* at HI further decreased the photosynthetic activity and vitality of plants. $P_N$ was extremely vulnerable by HI exposure—a 30 % reduction of leaf water content led to 80 %

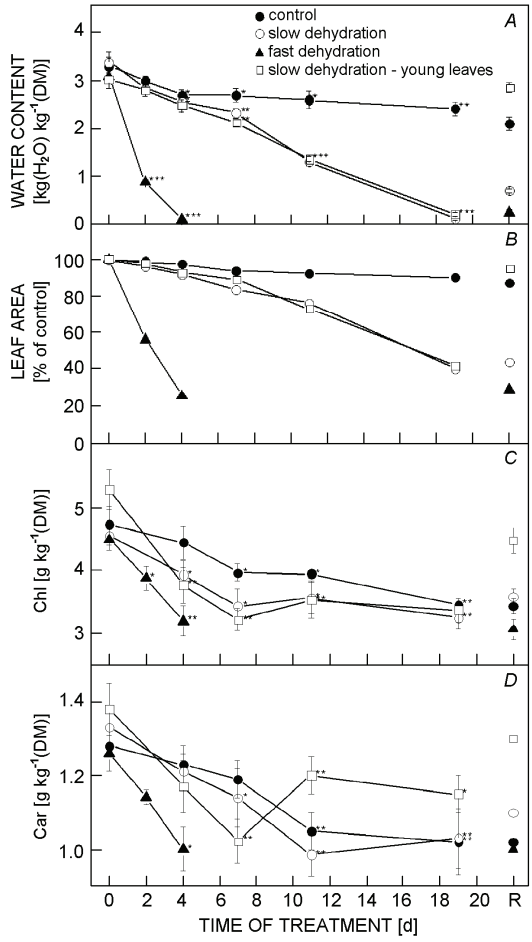


Fig. 1. Water content, leaf area, and contents of total chlorophyll (Chl) and carotenoids (Car) of mature leaves of *Haberlea rhodopensis* without (control) and with slow (SD) and fast (FD) desiccation under high irradiance (350 μmol $m^{-2}$ $s^{-1}$) during 4 d (FD) or 19 d (SD), respectively. The effect of SD on young leaves is shown as separate line. Data after 7 d recovery are given at the end of the x-axis (R). Means of 6 replications with standard errors. The levels of significant difference as compared with the onset of the treatment: $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$.

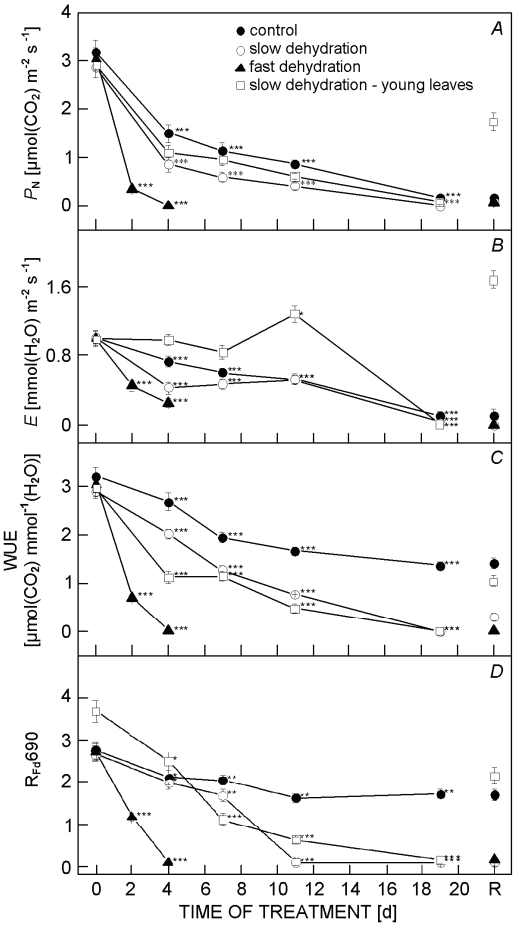


Fig. 2. Rates of net photosynthesis ($P_N$) and transpiration ($E$), water use efficiency (WUE), and fluorescence decrease ratio ($R_{Fd}$) of mature leaves of *Haberlea rhodopensis* without (control) and with slow (SD) and fast (FD) desiccation under high irradiance (350 μmol $m^{-2}$ $s^{-1}$) during a period of 4 d (FD) and 19 d (SD), respectively. The effect of SD on young leaves is shown as separate line. Data after 7 d of recovery are given at the end of the x-axis (R). The parameters were determined after 30-min irradiation when the leaves had reached a steady state. Means of 6 replications with standard error. The level of significant difference as compared with the onset of the treatment: $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$.

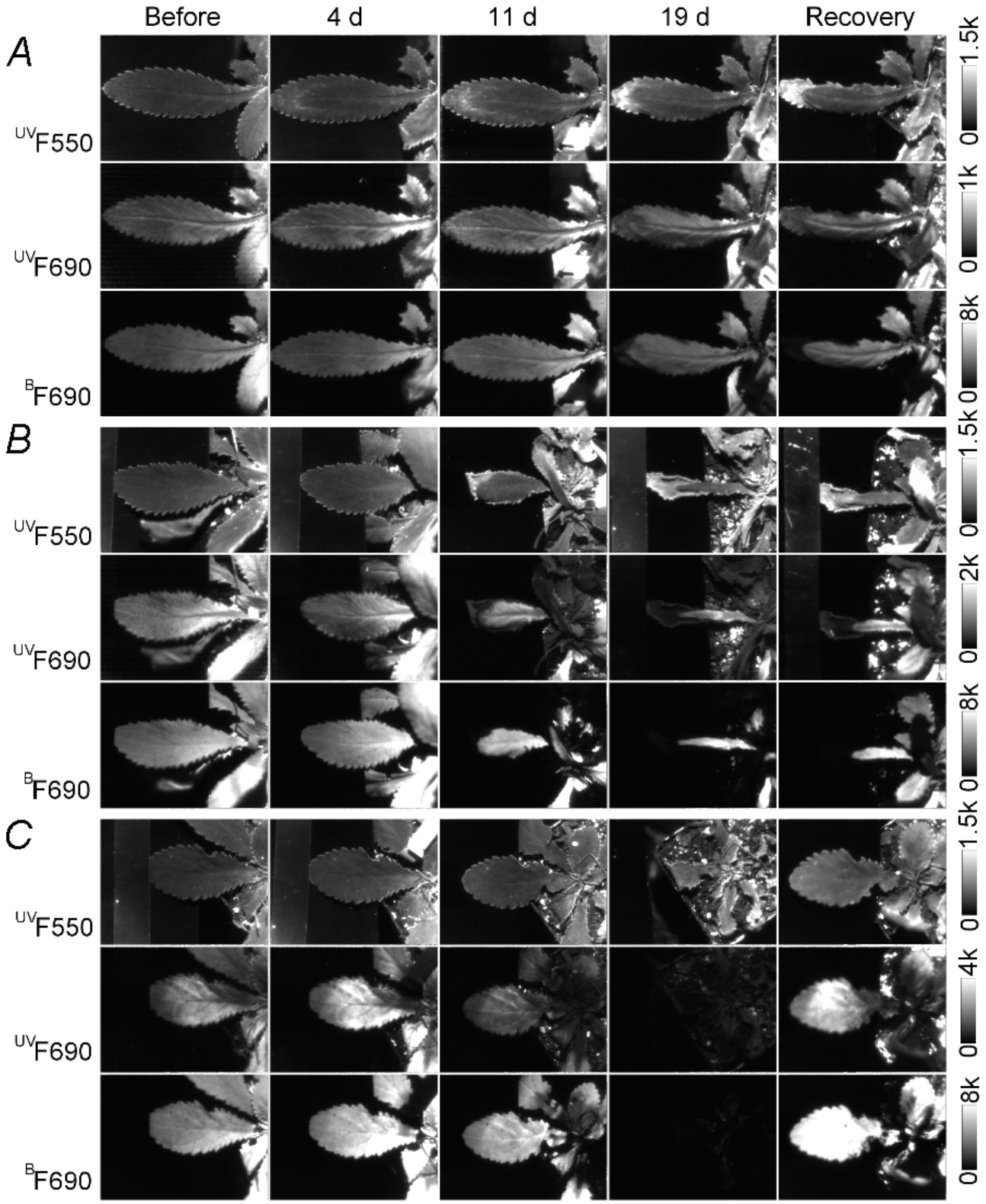


Fig. 3. Images of *Haberlea rhodopensis* for the green fluorescence ($^{UV}$F550), the UV-excited red fluorescence ($^{UV}$F690) and the blue-excited red fluorescence ($^{B}$F690) taken before and after 4, 11, and 19 d of desiccation as well as 7 d of recovery: (*A*) without desiccation (control), (*B*) slow desiccation (SD) of mature leaves under high irradiance, HI (350 μmol $m^{-2}$ $s^{-1}$) showing no real recovery, (*C*) SD of the youngest leaves under HI showing full recovery.

decline in $P_N$ (Fig. 2*A*). In contrast, our previous investigations showed that higher degree of desiccation (50 %) applied at low irradiance (30 μmol $m^{-2}$ $s^{-1}$) led to 50 % inhibition of $P_N$ (Georgieva *et al*. 2007). Decrease of $P_N$ has been related to limited $CO_2$ diffusion to the intercellular spaces of the leaf as a consequence of the reduced stomatal opening imposed by the water deficit and/or to impaired metabolism by direct inhibition of photochemical process (Chaves 1991, Cornic 2000). Under mild drought (30 % reduction in water content of SD-plants, Fig. 1*A*) the decline of $P_N$ was largely dependent on stomatal closure, which restricted water loss through transpiration (Fig. 2*B*). In the beginning of desiccation of *Haberlea* plants, $P_N$ declined more rapidly than photochemical activity as monitored by the $R_{Fd}$-values (Figs. 2*A*,*D*). The latter was strongly inhibited when leaf water content decreased by more than 60 % (Figs. 1 and 2). When $P_N$ is decreased at low water content, the electron transport becomes strongly reduced and electron transfer to oxygen increases, producing active oxygen species (Lawlor and Cornic 2002). The major source of oxidative damage during desiccation is probably associated with the absorption of excess photon energy (Alpert 2000). After inactivation of electron transport by desiccation, photon energy continues to be absorbed by photosynthetic pigments.

*H. rhodopensis* belongs to the homoiochlorophyllous type of desiccation tolerant plants that keep their Chl content during drying. The high amount of Chl molecules retained during desiccation could be a source for potentially harmful singlet oxygen production and may be a reason for irreversible damage of the photosynthetic apparatus during desiccation of *Haberlea* plants at HI.

According to Bewley (1979), desiccation-tolerant plants must be able to limit the damage to a repairable level, maintain the physiological integrity in the dried state, and mobilize mechanisms upon rehydration that repair damage suffered during desiccation and subsequent rehydration. The protective mechanisms against desiccation damage are generally induced during drying (Oliver *et al*. 1998, Tuba *et al*. 1998). The time needed for the accumulation of protective components like sugars and proteins is thought to be the reason why desiccation-tolerant plants do not survive rapid water loss (Oliver *et al*. 1998). However, the present study indicated that desiccation of *Haberlea* plants at HI, regardless of drying rate, caused viability loss of older leaves.

Curling of leaves during desiccation is an effective mechanism for decreasing the photon flux captured. Farrant *et al*. (2003) have shown that *C. wilmsii* did not survive drying under irradiation if the leaves were prevented from folding, despite protection from increased anthocyanin and sucrose and elevated antioxidant activity. The extensive shrinkage and folding of *Haberlea* leaves during both rapid and slow desiccation was not enough to avoid light-induced damages.

The fluorescence images (Fig. 3) clearly show the changes of size and shape of the leaves during desiccation and subsequent recovery. In some cases the Chl fluorescence was not homogeneously distributed over the leaf area, in particular the leaf veins showed a somewhat lower Chl fluorescence. Although this inhomogeneous pattern may not be of importance for the interpretation of this experiment, the fluorescence images allow the quantification of the fluorescence signals with a higher statistical confidence (Buschmann and Lichtenthaler 1998).

During desiccation of *Haberlea* leaves the blue and green fluorescence emission increased (Fig. 3 and Table 1). The blue and green fluorescence has been generally attributed to cell wall bound ferulic acid (a cinnamic acid), although the contribution of flavonoids and other simple phenols, such as coumaric acid, has also been suggested (Lichtenthaler and Schweiger 1998, Apostol *et al*. 2003). The contribution of flavonoid compounds to the blue and green fluorescence was confirmed using the mutant deficient in flavonoids (Hideg *et al*. 2002).

Table 1. Fluorescence parameters of mature leaves of *Haberlea rhodopensis* without (control) and with slow desiccation (SD) and subsequent recovery in HI as calculated from the leaf pixels of the fluorescence images (examples shown in Fig. 3*A*,*B*). The parameters are given as mean values with standard error. *The values for the fluorescence at 690 nm excited in the blue ($^{B}$F690) have been acquired with a gain about 3 times lower than those excited in the UV ($^{UV}$F690).

| Plants | | Before SD | 4 d SD | 7 d SD | 11 d SD | 19 d SD | 7 d recovery |
|---|---|---|---|---|---|---|---|
| Control | $^{UV}$F440 | 545±26 | 586±35 | 676±39 | 762±40 | 760±14 | 867±49 |
| | $^{UV}$F550 | 352±35 | 392±45 | 487±44 | 521±39 | 537±17 | 587±31 |
| | $^{UV}$F690 | 422±77 | 462±71 | 560±109 | 592±113 | 484±85 | 513±181 |
| | $^{B}$F690* | 2510±119 | 2498±87 | 3327±102 | 3413±233 | 3046±273 | 3198±519 |
| | $^{UV}$F440/$^{UV}$F690 | 1.39±0.20 | 1.38±0.25 | 1.38±0.30 | 1.46±0.31 | 1.72±0.29 | 1.89±0.57 |
| | $^{UV}$F550/$^{UV}$F690 | 0.90±0.16 | 0.93±0.20 | 0.98±0.22 | 1.00±0.23 | 1.22±0.22 | 1.28±0.39 |
| Desiccated | $^{UV}$F440 | 612±55 | 650±49 | 818±55 | 1128±181 | 1190±134 | 1288±90 |
| | $^{UV}$F550 | 388±46 | 414±36 | 507±51 | 748±141 | 795±120 | 913±94 |
| | $^{UV}$F690 | 682±137 | 557±101 | 580±128 | 410±123 | 285±58 | 374±73 |
| | $^{B}$F690* | 3415±451 | 3344±407 | 3727±446 | 2369±895 | 1463±543 | 1669±424 |
| | $^{UV}$F440/$^{UV}$F690 | 0.98±0.13 | 1.24±0.17 | 1.62±0.35 | 3.87±1.44 | 4.75±1.14 | 3.80±0.71 |
| | $^{UV}$F550/$^{UV}$F690 | 0.62±0.09 | 0.80±0.13 | 1.02±0.25 | 2.60±0.99 | 3.22±0.88 | 2.71±0.59 |

Even the smaller increase in blue and green fluorescence in drought-stressed barley as compared to untreated material suggested increased polyphenol synthesis, since drought stimulates the accumulation of polyphenols (Hideg *et al*. 2002).

The red Chl fluorescence detected at 690 nm was much higher when excited in the blue spectral region than in UV (Fig. 3 and Table 1). Taking the difference in gain voltage into account would increase the signal excited in the blue by a factor of about 20 over that excited in the UV. The higher Chl fluorescence with blue excitation is due to the facts (*a*) that Chl absorption is higher in the blue than in the UV (*e.g*. Rabinowitch 1951) and (*b*) that Chl molecules are located in the chloroplasts of the mesophyll (with exception of some chloroplasts of the guard cells in the epidermis covering the mesophyll on both leaf sides). UV absorbing substances in the epidermis shield the leaf tissue below from UV damage of the Chl containing mesophyll (Bilger *et al*. 2001, Lenk and Buschmann 2006).

Parallel to the increase of the blue and green fluorescence, the red Chl fluorescence—independent of its excitation wavelength—decreases strongly during the desiccation of *Haberlea* leaves (Fig. 3 and Table 1). Usually, the decreases of Chl fluorescence are interpreted in terms of an increase in photosynthetic activity and *vice versa* (see *e.g*. Buschmann 1981). But in case of the desiccation of the leaf tissue the optics of the leaves are changed. Due to reduction of leaf area (Fig. 1) and the shrinking of the cells and of the intercellular air spaces, the penetration of incident excitation radiation and of the emitted fluorescence is hampered. This is of particular importance for the fluorescence of the Chl molecules located more deeply inside the leaf than the blue emitting substances mainly located in the epidermis (Buschmann and Lichtenthaler 1998). Another argument for the changed leaf optics during desiccation of the *Haberlea* leaf is the decreased Chl fluorescence with reduced Chl content. A decrease in Chl content is usually accompanied by an increase of Chl fluorescence due to the better penetration of exciting radiation and the reduced re-absorption of emitted Chl fluorescence on its way to the leaf surface (Gitelson *et al*. 1998, Buschmann 2007).

In summary, our results showed that the shady desiccation-tolerant plant *H. rhodopensis* was very sensitive to HI treatment. Exposure of plants to 350 μmol $m^{-2}$ $s^{-1}$ for 19 d resulted in decreased content of photosynthetic pigments and $R_{Fd}$ as well as almost complete inhibition of $P_N$. Desiccation of *Haberlea* plants at HI caused irreversible damage of older leaves and only the youngest leaves survived and recovered after rehydration.

## References


Alpert, P.: The discovery, scope, and puzzle of desiccation tolerance in plants. – Plant Ecol. **151**: 5-17, 2000.

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

Barócsi, A., Kocsányi, L., Várkonyi, S., Richter, P., Csintalan, Z., Szente, K.: Two-wavelength, multipurpose, truly portable chlorophyll fluorometer and its application in field monitoring of phytoremediation. – Meas. Sci. Technol. **11**: 717-729, 2000.

Bewley, J.D.: Physiological aspects of desiccation tolerance. – Annu. Rev. Plant Physiol. **30**: 195-238, 1979.

Bilger, W., Johnsen, T., Schreiber, U.: UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. – J. exp. Bot. **52**: 2007-2014, 2001.

Buschmann, C.: The characterization of the developing photosynthetic apparatus in greening barley leaves by means of (slow) fluorescence kinetic measurements. – In: Akoyunoglou, G. (ed.): Photosynthesis. Vol. V. Pp. 417-426. Balaban International Science Services, Philadelphia 1981.

Buschmann, C.: Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves. – Photosynth. Res. **92**: 261-271, 2007.

Buschmann, C., Lichtenthaler, H.K.: Principles and characteristics of multi-colour fluorescence imaging of plants. – J. Plant Physiol. **152**: 297-314, 1998.

Chaves, M.M.: Effects of water deficits on carbon assimilation. – J. exp. Bot. **42**: 1-16, 1991.

Cornic, G.: Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. – Trends Plant Sci. **5**: 187-188, 2000.

Farrant, J.M., Vander Willigen, C., Loffell, D.A., Bartsch, S., Whittaker, A.: An investigation into the role of light during desiccation of three angiosperm resurrection plants. – Plant Cell Environ. **26**: 1275-1286, 2003.

Gaff, D.F.: Responses of desiccation tolerant 'resurrection' plants to water stress. – In: Kreeb, K.H., Richter, H., Hinckley, T.M. (ed.): Structural and Functional Responses to Environmental Stresses: Water Shortage. Pp. 255-268. SPB Academic Publishing, The Hague 1989.

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

Georgieva, K., Szigeti, Z., Sarvari, E., Gaspar, L., Maslenkova, L., Peva, V., Peli, E., Tuba, Z.: Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during desiccation and rehydration. – Planta **225**: 955-964, 2007.

Gitelson, A.A., Buschmann, C., Lichtenthaler, H.K.: Leaf chlorophyll fluorescence corrected for re-absorption by means of absorption and reflectance measurements. – J. Plant Physiol. **152**: 283-296, 1998.

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

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in

higher plants. – Plant Cell Environ. **25**: 275-294, 2002.

Lenk, S., Buschmann, C.: Distribution of UV-shielding of the epidermis of sun and shade leaves of the beech (*Fagus sylvatica* L.) as monitored by multi-colour fluorescence imaging. – J. Plant Physiol. **163**: 1273-1283, 2006.

Lichtenthaler, H.K.: Chlorophylls and carotenoids – the pigments of photosynthetic biomembranes. – In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. **148**. Pp. 350-382. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto 1987.

Lichtenthaler, H.K., Rinderle, U.: The role of chlorophyll fluorescence in the detection of stress conditions in plants. – CRC crit. Rev. anal. Chem. **19**: S29-S85, 1988.

Lichtenthaler, H.K., Schweiger, J.: Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants. – J. Plant Physiol. **152**: 272-282, 1998.

Muslin, E.H., Homann, P.H.: Light as a hazard for the desiccation-resistant 'resurrection' fern *Polypodium polypodioides* L. – Plant Cell Environ. **15**: 81-89, 1992.

Oliver, M.J., Wood, A.J., O'Mahony, P.: "To dryness and beyond" – preparation for the dried state and rehydration in vegetative desiccation-tolerant plants. – Plant Growth Regul. **24**: 193-201, 1998.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Rabinowitch, E.I.: Photosynthesis and Related Processes. Vol. II, Part 1. – Interscience Publishers, New York – London 1951.

Rasband, W.S.: Image, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2008.

Sherwin, H.W., Farrant, J.M.: Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. – Plant Growth Regul. **24**: 203-210, 1998.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficits and desiccation. – New Phytol. **125**: 27-58, 1993.

Tuba, Z., Proctor, M.C.F., Csintalan, Z.: Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: a comparison and an ecological perspective. – Plant Growth Regul. **24**: 211-217, 1998.