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Recovery of photosynthetic activity of resurrection plant *Haberlea rhodopensis* from drought- and freezing-induced desiccation

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Abstract

The recovery of photosynthetic activity during rehydration of *Haberlea rhodopensis* from drought- and freezing-induced desiccation were investigated. The water uptake during the initial 15 h was slow thus preventing cellular damages. The results showed faster recovery of quantum efficiency of PSII in plants rehydrated after freezing stress (RAF) compared to plants rehydrated after drought stress (RAD) and the most significant differences between them were evident after 9–15 h of rehydration. Following rehydration, PSI activity recovered faster compared to PSII and in contrast to PSII, its activity was higher in RAD compared to RAF plants. During the first hours of rehydration, prominent alterations in energy transfer between photosynthetic complexes occurred as revealed by 77 K fluorescence of isolated thylakoids. High proportion of thermal energy dissipation in dry plants and during the first hours of rehydration protects them from photooxidation; the role of PSII reaction center quenching during the recovery was suggested.

Additional key words: 77 K fluorescence spectra; chlorophyll fluorescence; desiccation tolerance.

Introduction

In natural habitats, plants are constantly exposed to various kinds of environmental stresses that adversely affect their growth and productivity. In addition, the ongoing climate changes are known to have an enormous impact on current plant diversity. That is why it is very important to unravel the mechanisms that plants have evolved to cope with stress conditions. Understanding plant responses to environmental constraints is important for identifying strategies to improve tolerance to abiotic stresses in crop species and for predicting the ecological distribution of natural vegetation under the projected climate changes.

Resurrection plants are unique among vascular angiosperms with their ability to survive desiccation to air-dry state. Desiccation tolerance of vegetative tissue is uncommon phenomenon and consists in losing more than 95% of the protoplasmic water or 'the ability to survive drying to, or below, the absolute water content of 0.1 g(H_2O) g^{-1}(dry mass), this being equivalent to air-dryness at 50% relative humidity and 20°C and corresponding to a water potential of ≤ –100 MPa' (Vertucci and Farrant 1995). Only 300 species of resurrection plants are reported and most of them are living in the southern hemisphere – Africa, America, and Australia (Gaff and Oliver 2013). One family of resurrection plants is distributed in the northern hemisphere (Europe and Asia) – Gesneriaceae. Five species from three genera are located in Europe (*Haberlea rhodopensis*, *Ramonda serbica*, *Ramonda nathaliae*, *Ramonda myconi*, and *Jankaea heldreichii* (Rakić *et al*. 2014, Georgieva *et al*. 2017a, Gashi *et al*. 2019, Fernández-Marín *et al*. 2020). *Boea hygrometrica*, *Paraboea rufescens*, and few other species could be found in Asia (Huang *et al*. 2012, Liu *et al*. 2019). *Haberlea rhodopensis* is a perennial herbaceous rock poikilohydric plant, forming dense tufts of leaves, every rosette bearing in spring one to five flower-stalks. This Tertiary relict emerged in the late Oligocene (Petrova *et al*. 2015) and is characterized with a high ecological plasticity, growing at altitude from 136 m to near 1600 m at different temperature, water, and light conditions (Daskalova *et al*. 2011). Resurrection plants are excellent model systems for studying the mechanisms

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Abbreviations: (1 – F_v'/F_m') – the relative proportion of the energy absorbed and dissipated as heat in the PSII antennae; (1 − q_P) – excitation pressure; Chl – chlorophyll; E_{470}/E_{436} – the ratio of intensities of bands at 470 and 436 nm in the excitation spectra of fluorescence emitted at 685 nm; E_{680}/E_{650} – the ratio of intensities of bands at 680 and 650 nm in excitation spectra of fluorescence emitted at 735 nm; F_{685}/F_{695} − the ratio of intensities of fluorescence bands at 685 and 695 nm; F_{735}/F_{685} − the ratio of intensities of fluorescence bands at 735 and 685 nm; F_v/F_m – maximal quantum yield of PSII photochemistry; RAD – rehydrated after drought stress; RAF – rehydrated after freezing stress; RWC – relative water content; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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underlying desiccation tolerance. Desiccation-tolerant plants must be able to limit the damage to a repairable level, maintain physiological integrity in the dried state, and mobilize mechanisms upon rehydration that repair damages during desiccation and subsequent rehydration (Bewley 1979, Oliver *et al*. 2000). Resurrection plants tolerate desiccation through a set of different mechanisms, including the downregulation of metabolism, upregulation of housekeeping and novel antioxidants to minimize free radical damage, subcellular reorganization to minimize mechanical damage associated with turgor loss, accumulation of specific proteins, and maintenance of the structure and function of macromolecules through the accumulation of disaccharides and other compatible compounds (for review *see* Farrant *et al*. 2007, Morse *et al*. 2011, Giarola *et al*. 2017, Liu *et al*. 2019). 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). Most of the studies on these plants have focused on the characterization of protection mechanisms in the dehydration phase and thus the rehydration processes of resurrection plants have been scarcely investigated (Giarola and Bartels 2015). In fact, most of the researches are focused on later stages of rehydration when plants regain most of their water content. However, a rapid water uptake upon rehydration is a serious stress factor for plants which could induce cellular damages (Oliver *et al*. 2000). Several activities are necessary during rehydration to reestablish the original cellular organization and metabolic activities. These mechanisms are part of the survival strategy of resurrection plants and must be coordinated to permit successful recovery from desiccation.

Photosynthesis is one of the most sensitive processes to environmental stress conditions. The loss of photosynthetic capacity could be triggered by several factors, such as the closure of stomata, degradation of pigments, loss of photosynthetic function or destruction of photosynthetic structure (Deng *et al*. 2003, Peeva and Cornic 2009). *H. rhodopensis* belongs to the group of homoiochlorophyllous resurrection plants which retain most of the chlorophyll (Chl) and maintain their photosynthetic apparatus during desiccation, allowing them to resume photosynthetic activity quickly upon water availability (Georgieva *et al*. 2007, 2009). However, the high concentration of Chl during desiccation is a source for the production of harmful reactive oxygen species that can induce oxidative stress (Lawlor and Cornic 2002). Thus, preserving the integrity of photosynthetic apparatus is of primary importance for recovery of plants after stress treatment (Georgieva and Mihailova 2016).

In contrast to the most resurrection plants, *H. rhodopensis* is exposed to and can withstand freezing temperatures during winter. The first data available in the literature about tolerance of resurrection plants (*R. myconi*) to low temperatures are from Kappen (1966). Recently, Fernández-Marín *et al*. (2018, 2020) showed that *R. myconi* can tolerate freezing temperatures during winter. The effect of short-term low temperature treatment (3 d at 4°C) on the metabolite changes in *H. rhodopensis* was investigated (Benina *et al*. 2013). Our previous studies showed that when the night temperatures drop down to –2 to –4°C, leaf rolling starts but with the rise of temperature during the day leaves unfold (Georgieva *et al*. 2018). However, exposure of plants to about –10°C resulted in a reduction in relative water content of leaves and thereafter they desiccated very quickly to air-dry state (Georgieva *et al*. 2018). Thus, similar to drought, freezing stress also causes dehydration of plants and they survive the harsh winter conditions in the dry state (Daskalova *et al*. 2010).

Understanding the response of resurrection angiosperms to dehydration and rehydration is critical for deciphering the mechanisms of how plants cope with the rigors of water loss from their vegetative tissues. For this reason, the investigations of rehydration phenomenon in *H. rhodopensis*, especially after the onset of rewatering of dried plants, are necessary. In addition, the comparison of mechanisms of recovery from drought- and freezing-induced desiccation will contribute for better understanding the strategies for survival of this resurrection plant.

The aim of present study was to investigate and compare the recovery of photosynthetic activity during rehydration of *Haberlea rhodopensis* plants from drought- and freezing-induced desiccation. The functional activity of photosynthetic apparatus was estimated by measuring the photochemical efficiency of PSII and PSI, the energy interaction between them, and the content of photosynthetic pigments.

Materials and methods

Desiccation and rehydration of plants: *Haberlea rhodopensis* Friv. tufts of shade ecotype were initially collected from the Rhodope Mountains and further cultivated in pots with peat soil (*Stender*, Schermbeck, Germany) under *ex situ* (outdoor) environmental conditions at least for one year. Part of the plants were transferred to a climatic chamber *FytoScope FS 130* (*Photon Systems Instruments*, Drásov, Czech Republic) and kept at 20/18°C day/night temperature, 60% humidity, 12-h photoperiod, and irradiance of 25 μmol(photon) $m^{-2} s^{-1}$ for two weeks. Then plants were subjected to drought stress by withholding irrigation and desiccated to air-dry state. Desiccation was performed during summer (July–August 2019) and plants were rehydrated in August–September to follow natural processes. Before rehydration, the plants were kept in air-dry state for two weeks. The other plants were left outdoor and were exposed to cold and freezing temperatures in natural conditions during autumn and winter (November 2018–February 2019). The light intensity during the experiment was 30–60 μmol(photon) $m^{-2} s^{-1}$. Low positive temperatures did not influence the relative water content (RWC) of leaves. But when the temperature dropped to about –10°C, plant's dehydration started and they were in air-dry state during the winter (about three months). Dry plants were taken from outside and their rehydration was performed in March similarly to the natural conditions.

Rehydration of plants desiccated to air-dry state as a result of drought or freezing stress was carried out in laboratory conditions at 21–23°C and light intensity of 25–30 µmol(photon) m^{-2} s^{-1}. At first the soil was well irrigated and then the pots were transferred in a modified desiccator where the desiccant at the bottom was replaced by water, providing permanent high humidity by a water pump. The measurements were conducted on dry leaves (0 h) and after 3, 5, 7, 9, 15, 24 h, and 7 d of rehydration.

Relative water content (RWC): The RWC of *H. rhodopensis* leaves was determined gravimetrically by weighing them before and after oven-drying at 80°C to a constant mass and expressed as percentage of water content in dehydrated tissue compared to water-saturated tissues, using the equation: RWC [%] = (FM – DM)/(TM – DM) × 100, where FM – fresh mass, DM – dry mass, and TM – turgid mass. TM was measured on leaves maintained for 12–16 h at 4°C in the dark floating on water.

Pigments: Chl *a*, Chl *b*, and total carotenoids were extracted from leaf disks with 80% acetone. The pigment content was determined spectrophotometrically by *Multiskan Spectrum* (*Thermo Fisher Scientific*, Waltham, Massachusetts, USA) using the equations of Lichtenthaler (1987). The data were calculated on a dry mass basis.

Chl *a* fluorescence induction: Chl *a* fluorescence induction was measured with a portable fluorometer *PAM-2500* (*Heinz Walz GmbH*, Effeltrich, Germany). The leaves were dark-adapted for 15 min and PAR of 90 µmol(photon) m^{-2} s^{-1} was used for the measurements. The temperature during measurements was 21–23°C. All used basic parameters were given by *PamWin-3* software (*Heinz Walz GmbH*, Effeltrich, Germany). The maximum efficiency of PSII photochemistry was calculated as F_v/F_m immediately after the predarkening period. The actual efficiency of PSII electron transport during illumination was estimated at steady state as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty *et al.* 1989), where F_m' is the maximum fluorescence and F_s is the steady-state fluorescence in light-adapted state. Excitation pressure of PSII, which gives an approximate measure of the reduction state of the first electron acceptor Q_A of PSII, was calculated as $1 - q_P$, as q_P is determined by the equation $q_P = (F_m' - F_s)/ (F_m' - F_0)$ (van Kooten and Snel 1990). The relative proportion of the energy absorbed and dissipated as heat in the PSII antennae (referred as thermal energy dissipation in the antenna) was estimated by $1 - (F_v'/F_m')$ (Demmig-Adams *et al*. 1996). A fraction of light absorbed in PSII antennae that was neither utilized in photosynthetic electron transport nor dissipated thermally, labelled as 'excess' energy was estimated using the equation: Excess = $F_v'/F_m' \times (1 - q_P)$ (Demmig-Adams *et al.* 1996).

P_{700} measurements: The redox state of P_{700} was monitored *in vivo* as ΔA_{820} nm absorption changes. A *Walz ED 700DW-E* emitter/detector unit was connected to a *PAM 101E* main control unit (*Heinz Walz GmbH*, Effeltrich, Germany). P_{700} was oxidized by far-red (FR) light from a photodiode (*FR-102*, *Heinz Walz GmbH*, 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. The temperature during measurements was 21–23°C.

Thylakoid preparation: Thylakoid membranes were prepared as described by Georgieva *et al*. (2009). Leaves were ground in liquid nitrogen to fine powder, resuspended in buffer A [50 mM HEPES (pH 7.5), 400 mM NaCl, 10 mM $MgCl_2$, 2 g L^{-1} bovine serum albumin (BSA), 10 mM dithionite, 4 g L^{-1} ascorbate] and centrifuged at 8,600 × *g* for 5 min and 4°C. The pellet was resuspended in buffer A again and centrifuged at 8,600 × *g* for 10 min, 4°C. The sample was filtered through two layers of *Miracloth* (*Merck Millipore*, Darmstadt, Germany) and cotton wool. The filtrate was centrifuged at 8,600 × *g* for 10 min, 4°C and the resulted pellet resuspended in 50 mL buffer B [50 mM MES (pH 6.0), 150 mM NaCl, 5 mM $MgCl_2$, 1 g L^{-1} BSA, 0.5 g L^{-1} ascorbate]. Following centrifugation step at 8,600 × *g* for 10 min, 4°C, the pellet was resuspended in 150 µL buffer C (50 mM MES, pH 6.0, 5 mM $MgCl_2$, 15 mM NaCl). The Chl content of thylakoid fractions was determined spectrophotometrically in 80% acetone by *Multiskan Spectrum* (*Thermo Fisher Scientific*, Waltham, Massachusetts, USA) using the equations of Lichtenthaler (1987).

Steady-state low temperature (77 K) fluorescence spectra: Low temperature (77 K) fluorescence emission and excitation spectra of thylakoid membranes were registered by a *Jobin Yvon JY3* spectrofluorometer (*Division d'Instruments S.A*., Longjumeau, France), equipped with a low temperature device and a red sensitive photomultiplier as described in Velitchkova and Popova (2005). Samples from isolated thylakoid membranes at concentration of 10 µg Chl (*a*+*b*) ml^{-1} were transferred into a quartz tube for fluorescence measurement and immediately frozen in liquid nitrogen. Emission spectra were recorded in the region of 660–780 nm under excitation with 436 nm. Excitation spectra for emission at 735 nm (PSI) were recorded in the red (700–610 nm) region and excitation spectra of emission from PSII (at 685 nm) in the blue region (500–410 nm). The width of the emission and excitation slits was 4 nm. The spectra were analyzed by *Origin 6.0* (*OriginLab Co*., Northampton, MA, USA).

Statistical analysis: Two rehydration cycles were performed for each group of plants and the measurements of RWC, Chl *a* fluorescence, and ΔA_{820} nm absorption changes were repeated three times per cycle using leaves from different plants (n = 6). Thylakoids were isolated from 10 g of leaves sampled from different plants per each time point during rehydration. Comparison of means was made by the *Fisher*'s least significant difference (LSD) test at $P \leq 0.05$ following analysis of variance (*ANOVA*). A statistical software package (*StatGraphics Plus*, version *5.1* for *Windows*, USA) was used. The *Pearson*'s correlation coefficient (r) was used to measure the strength of a linear association between two variables. It was calculated in *Microsoft Excel*. The formulas return a value between –1 and 1, where 1 indicates a strong positive relationship and –1 indicates a strong negative relationship. A result of zero indicates no relationship at all.

Results

Recovery of relative water content: Measurements of RWC during recovery of *H. rhodopensis* from drought- and freezing-induced desiccation showed that water uptake during the initial 15 h was slow, but thereafter the rate of rehydration increased (Fig. 1). Indeed, high correlation coefficient of *Pearson* (r = 0.995) was determined for the changes in RWC upon rehydration of both groups of plants. Some differences were observed after 9–24 h of rehydration, when the RWC values of leaves recovering after freezing-induced desiccation (RAF) were higher than those of leaves recovering after drought stress (RAD). RWC reached 14 and 22% after 15 h of rehydration of RAD and RAF plants, respectively, but it increased up to 45 and 70% after 24 h. There were no significant differences in RWC of both plant groups after 7 d of rehydration, when they reached the usual water content of completely rehydrated plants (90%).

Pigment content: Being homoiochlorophyllous resurrection plant, *H. rhodopensis* preserves most of its Chl content both during desiccation and rehydration. Some reduction in the Chl content of RAD plants was observed during the first 24 h of rehydration (Fig. 2*A*). The Chl content of dry RAF plants (0 h) was lower compared to dry RAD plants but upon rehydration their Chl content started increasing after 7 h of rehydration. Similar changes were detected for the carotenoid content in both plant groups during rewatering (Fig. 2*B*).

Chl *a* fluorescence: Upon rehydration, the maximum quantum efficiency of PSII photochemistry, estimated by the ratio F_v/F_m, gradually increased but it was statistically significant after 7 h of rehydration in both plant groups (Fig. 3). In agreement with the higher RWC, the values of F_v/F_m measured after 9 and 15 h of rehydration were higher in RAF plants. A significant enhancement of F_v/F_m was detected after 24 h of rehydration, reaching the values typical for fully hydrated plants after 7 d of rehydration. It should be mentioned that there were no differences in quantum efficiency of PSII between both plant groups after 24 h and 7 d of rehydration. The changes in the fractions of light absorbed by PSII antennae allocated to PSII photochemistry and thus photosynthetic electron transport, Φ_{PSII} (*dark grey*), thermal energy dissipation, $1 - F_v'/F_m'$ (*light grey*) and 'excess' energy, which was neither utilized in photosynthetic electron transport nor thermally dissipated, $F_v'/F_m' \times (1 - q_P)$ (*grey*) during rehydration of plants are presented in Fig. 4. In contrast to F_v/F_m, the quantum yield of PSII electron transport (Φ_{PSII}) recovered more slowly in the course of rehydration. The changes in Φ_{PSII} confirmed the faster recovery of RAF plants. Our results show that a significant enhancement in the photochemical activity of PSII of RAF plants was observed after 9 h of rehydration, when the values of Φ_{PSII} increased up to 25% of the level of completely rehydrated plants. The electron transport through PSII of RAD plants sharply increased after 24 h of rehydration. In fact, the extent of recovery of Φ_{PSII} in both plant groups after 24 h of rehydration was similar.

The low photochemical activity of PSII during the first 7 h of rehydration (Fig. 4) was accompanied by an increased amount of closed PSII reaction centers, estimated

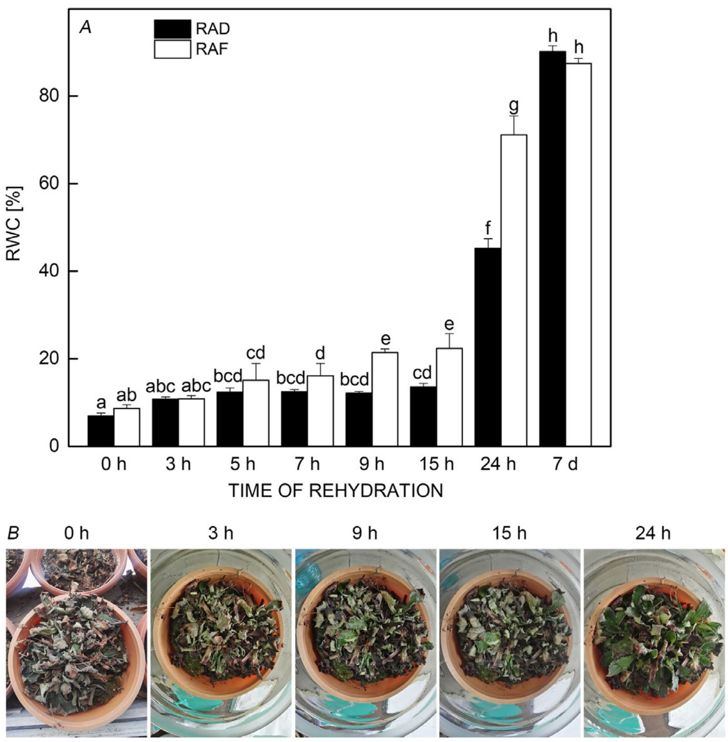

Fig. 1. Changes in relative water content (RWC) after rehydration of *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation (*A*) and plants appearance after rehydration of RAF plants (*B*). The error bars show SE and data represent the mean of n = 6; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

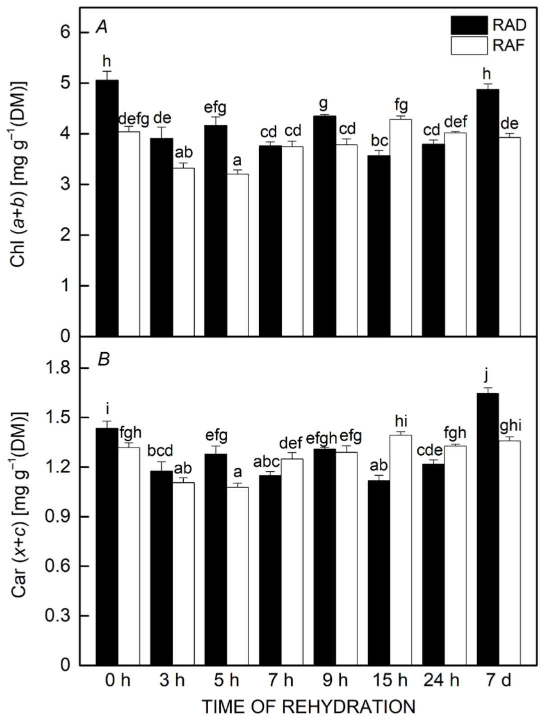

Fig. 2. Changes in leaf chlorophyll [Chl (*a*+*b*)] (*A*) and carotenoid [Car (*x*+*c*)] (*B*) content after 3, 5, 7, 9, 15 and 24 h, and after 7 d of rehydration of *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of $n = 6$; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

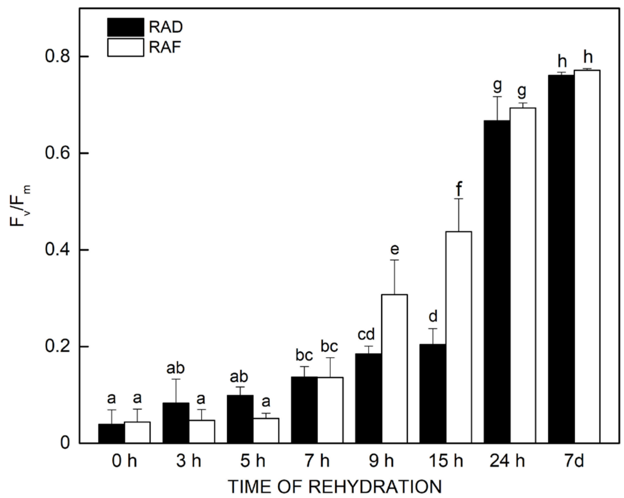

Fig. 3. Maximum quantum yield of PSII photochemistry in the dark-adapted state, F_v/F_m of rehydrated (3, 5, 7, 9, 15 and 24 h, and 7 d) *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of $n = 6$; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

by the changes in $1 - q_P$ or so-called excitation pressure (Fig. 5). Statistically significant reduction in the values of $1 - q_P$ was observed after 7 h, reaching the lowest values after 7 d of rehydration in both plant groups. Excitation pressure declined stronger in RAF plants compared to the RAD plants in the period 9–24 h of rehydration, which was in agreement with their higher PSII efficiency. A sharp reduction in excitation pressure was observed after 24 h of rehydration and the values in both plant groups after 7 d of rehydration were close to those for fully hydrated plants. In fact, high negative correlation coefficient of *Pearson* was determined for the rehydration-induced changes in the quantum yield of PSII electron transport and excitation pressure in both plant groups ($r = -0.993$ and $r = -0.965$ for RAD and RAF plants, respectively; $P \leq 0.05$).

Excitation pressure has been suggested to be a major prerequisite for the induction of efficient dissipation of the excess excitation energy thus protecting the PSII reaction center from over excitation. Indeed, the results showed that the fraction of light absorbed in PSII antennae, which is deactivated *via* thermal energy dissipation in the antennae, estimated as $1 - F_v'/F_m'$, was high at the first hours of

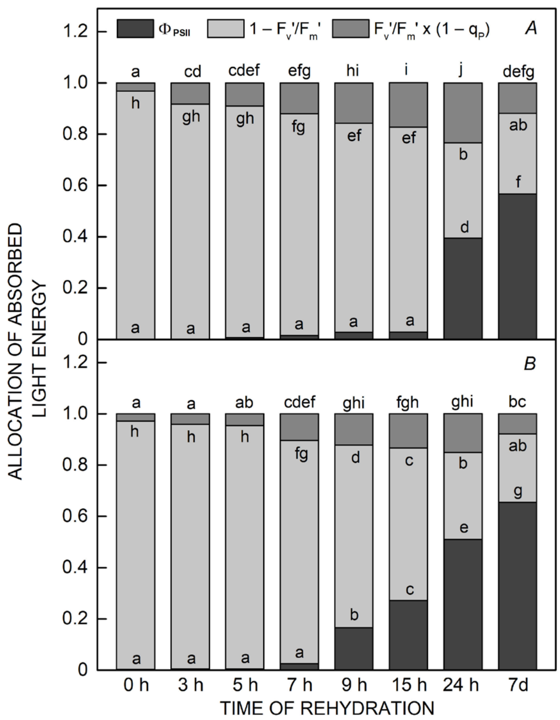

Fig. 4. Allocation of light absorbed by the PSII antennae to PSII photochemistry and thus photosynthetic electron transport, Φ_{PSII} (*dark grey*), thermal energy dissipation, $1 - F_v'/F_m'$ (*light grey*), and 'excess' energy, which represents a fraction of the absorbed light that was neither utilized in photosynthetic electron transport nor thermally dissipated, $F_v'/F_m' \times (1 - q_P)$ (*grey*) in *Haberlea rhodopensis* plants rehydrated from drought – (RAD, *A*) and freezing-induced (RAF, *B*) desiccation. The data represent the mean of $n = 6$; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

rehydration and it started declining after 7 h (Fig. 4, *light grey*). The values of 1 – F_v'/F_m' were higher in RAD plants after 9–15 h of rehydration and were similar to those in RAF plants after 24 h and 7 d. Actually, high correlation coefficient of *Pearson* was determined for the rehydration-induced changes in the excitation pressure and thermal energy dissipation in both plant groups (r = 0.990 and r = 0.982 for RAD and RAF plants, respectively; $P \leq 0.05$).

The levels of excess light that is not going into either photosynthetic electron transport or thermal dissipation, $F_v'/F_m' \times (1 - q_P)$, gradually increased during rehydration of both RAD and RAF plants, reaching maximum after 24 h (Fig. 4, *grey*). Then the values of $F_v'/F_m' \times (1 - q_P)$ significantly decreased in completely rehydrated plants.

P_{700}: The increase in leaf absorption in the 820-nm region (ΔA_{820}) after far-red irradiance reflected increased PSI activity which was accompanied with increased oxidized form of PSI reaction center P_{700}^+. Following rehydration, PSI activity recovered faster compared to PSII (Fig. 6). Surprisingly, in contrast to PSII, better recovery of PSI activity was observed in RAD plants compared to RAF ones. PSI reached maximum activity after 15 and 24 h of rehydration of RAF and RAD plants, respectively.

77 K fluorescence: In the present study we analyzed the changes in 77 K fluorescence spectra of isolated thylakoid membranes from RAD and RAF *H. rhodopensis* plants. Fluorescence spectra at low temperature (77 K) of isolated thylakoid membranes are characterized by emission bands at 685 and a shoulder at 695 nm, emitted by the complex of PSII and a band at 735 nm emitted by PSI (Krause and Weis 1991, Andrizhiyevskaya *et al*. 2005). The intensities of these bands depend on the populations of both photosystems, energy delivery to them and on the spillover of excited energy from PSII to PSI. On the base of analysis of emission spectra, the ratios of intensities of fluorescence bands at 685, 695, and 735 nm were calculated and presented (Fig. 7). The participation of different Chl pools in energy supply of both photosystems was estimated analyzing the excitation spectra of fluorescence at 735 nm (emitted by PSI) and at 685 nm (emitted by PSII) (Fig. 8).

The rehydration-induced alterations in the fluorescence ratio F_{735}/F_{685} at excitation with 436 nm (exciting preferably Chl *a*) are presented in Fig. 7*A*. The first 7 h of rehydration led to significant changes in the ratio F_{735}/F_{685}. Although the value for F_{735}/F_{685} was lower for RAD (1.35) than that for RAF (1.42) in fully desiccated plants, for RAD plants, it changed more considerably reaching a value of 1.7 after 3 h of rehydration. After 7 h of rehydration the values for both samples were very close but after 7 d (fully rehydrated plants), F_{735}/F_{685} ratio for RAD plants was higher than that for RAF.

Changes of ratio F_{685}/F_{695} (Fig. 7*B*) characterize alteration of energy interaction within PSII complexes as the peak at 685 nm is believed to be emitted by PSII core complex and the shoulder at 695 nm by CP47 (Andrizhiyevskaya *et al*. 2005). During the first 3–7 h of rehydration, a decrease of F_{685}/F_{695} was detected for both types of rehydrated plants – RAD and RAF.

In order to estimate any changes in the antenna complexes and energy supply of both photosystems, the excitation spectra of fluorescence emitted by the complexes of PSI (735 nm) and PSII (685 nm) were analyzed in the 'red' (700–610 nm) and 'blue' (500–410 nm) region, respectively. The values of E_{680}/E_{650} (excitation of PSI) for thylakoid membranes isolated from RAF plants were higher than that for RAD plants for all presented samples

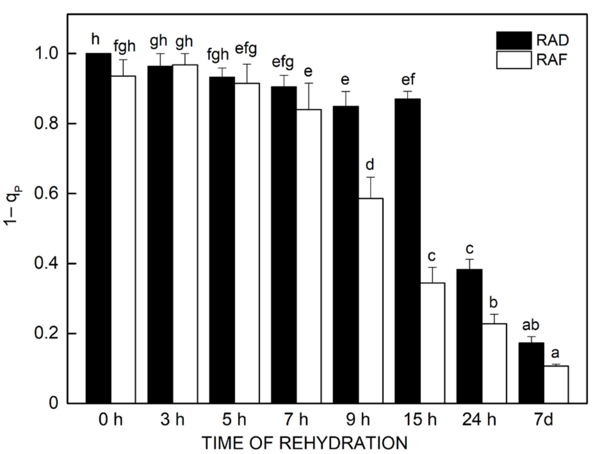

Fig. 5. Changes in excitation pressure, 1 – q_P measured in rehydrated (3, 5, 7, 9, 15, and 24 h, and 7 d) *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of n = 6; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

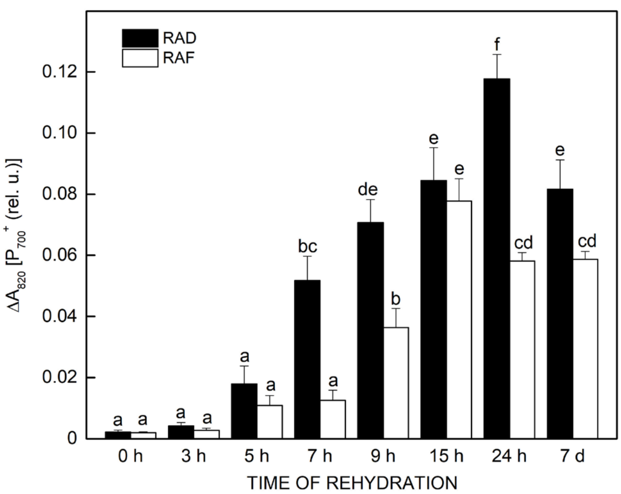

Fig. 6. Photochemical activity of PSI, evaluated by leaf absorption changes at ΔA_{820} after far-red illumination, after 3, 5, 7, 9, 15, and 24 h, and after 7 d of rehydration of *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of n = 6; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

except for those hydrated for 7 d. For thylakoid membranes isolated from both groups of plants (RAD and RAF), a decrease of ratio E_{680}/E_{650} was observed in the first 3 h of rehydration thus indicating an increase in involvement of Chl *b* molecules in comparison to Chl *a* in energy supply of PSI (Fig. 8*A*). At the same time period for RAD plants, an increase of E_{470}/E_{436} was observed for emission at 685 nm demonstrating an enhanced participation of Chl *b* in energy delivery to PSII (Fig. 8*B*). However, in the first 3–7 h, a decrease of E_{470}/E_{436} for emission at 685 nm was observed for RAF plants in contrast to RAD plants, thus showing different rearrangement of PSII and its antenna after rehydration of plants subjected to freezing-induced water deficit in comparison with desiccation induced by drought.

Discussion

In order to survive desiccation to air-dry state and to completely recover functional activity after rehydration, the homoiochlorophyllous resurrection plants have to preserve the integrity of photosynthetic apparatus upon desiccation. In order to reach this goal, plants have developed different mechanisms not only for diminishing damages suffered during severe water loss but during rehydration as well. It has been demonstrated that if the protective mechanisms during desiccation are hampered or inadequate then the importance of protective mechanisms at increased water availability is crucial for achieving plant vitality (Cooper and Farrant 2002).

In the present study, we investigated the recovery of photosynthetic activity after rehydration of *Haberlea rhodopensis* from air-dry state induced by drought or freezing stress for better understanding the strategies for survival of this resurrection plant. The results show that water uptake during the initial 15 h was slow, which could be considered as an adaptive defense mechanism to avoid cellular damages by a rapid water uptake upon rehydration. The plants increase their water content faster after 24 h of rehydration. Hence, the kinetics of recovery of RAD and RAF plants was similar and consists of two phases – slow and rapid. The investigations on recovery of resurrection plants after the onset of rewatering were performed mainly with genus *Ramonda*, close relative of *Haberlea rhodopensis* (Gesneriaceae). According to Rakić *et al*. (2014), at the beginning of rehydration, the recovering plant goes through an oscillatory, unstable, and vulnerable short period. The plant regains satisfactory stability in about 48 h but complete repair could be seen

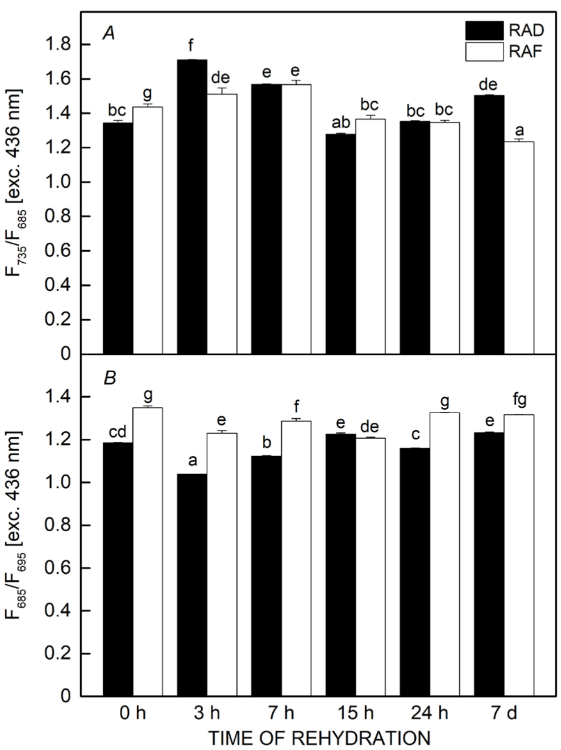

Fig. 7. Changes in the ratios of intensities of fluorescence bands at 735 and 685 nm, F_{735}/F_{685} (*A*) and 685 and 695 nm, F_{685}/F_{695} (*B*) after 3, 7, 15 and 24 h, and after 7 d of rehydration of *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of n = 6; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

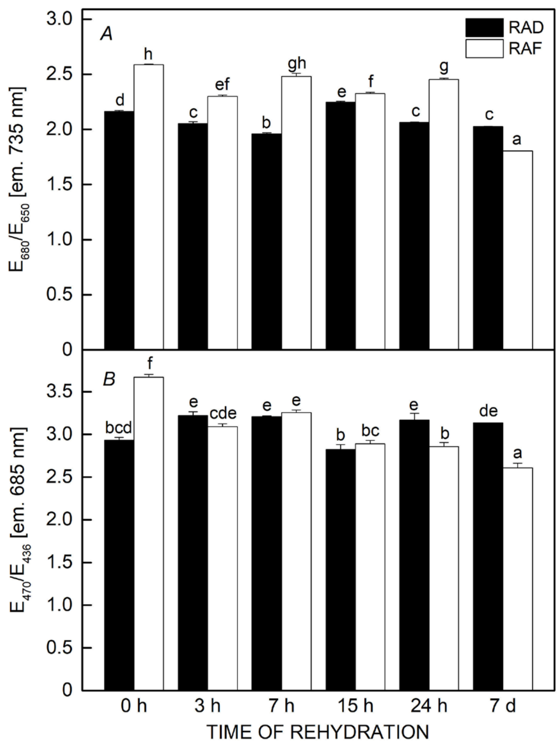

Fig. 8. Changes in the ratio of intensities of bands at 680 and 650 nm in excitation spectra of fluorescence emitted at 735 nm, E_{680}/E_{650} (*A*) and 470 and 436 nm in the excitation spectra of fluorescence emitted at 685 nm, E_{470}/E_{436} (*B*) after 3, 7, 15, and 24 h, and after 7 d of rehydration of *Haberlea rhodopensis* plants from drought- (RAD) and freezing-induced (RAF) desiccation. The error bars show SE and data represent the mean of n = 6; *the same letters* within a graph indicate no significant differences assessed by *Fisher*'s LSD test ($P \leq 0.05$) after performing *ANOVA*.

after 6 d of rehydration. Živković *et al.* (2005) showed that rehydration of desiccated *Ramonda serbica* for 6 h and 24 h increased their RWC up to 23 and 45%, respectively, which was similar to our results with RAD plants. However, the extent of increase of RWC during rehydration of dry plants varies in different studies even for the same plant species (Jovanović *et al.* 2011, Gashi *et al.* 2013). The time of recovery depends on plant size and age, method of rehydration, drying rate, desiccation extent, and duration before rehydration (Farrant *et al.* 1999, Farrant 2007). Gashi *et al.* (2013) demonstrated that the RWC was restored more rapidly in *Ramonda nathaliae* than in *Ramonda serbica* reaching 70 and 30%, respectively, after 6 h of rehydration. In addition, Tan *et al.* (2017) showed that completely dried plants *Boea hygrometrica* restored their RWC very fast upon rehydration reaching 50 and 95% RWC within only 12 and 24 h, respectively. Most probably, this rapid recovery was due to plant age since four-month-old plants propagated from seeds were used in the experiments. Very fast restoration of RWC was also observed after rehydration of *in vitro* propagated *H. rhodopensis* (Djilianov *et al.* 2011). Plants regain 56% of their RWC after 1–3 h of rewatering. *In vitro* propagated plants are small and it is well known that younger leaves restore their RWC faster. Our results show faster recovery of RAF compared to RAD *H. rhodopensis* plants which could be due to different environmental conditions during desiccation. In fact, previous studies on *H. rhodopensis* showed that the water uptake and the recovery of the roots were faster than leaves upon rewetting (Péli *et al.* 2012).

Significant enhancement in PSII activity was observed when RWC reached 20%. The results showed faster recovery of quantum efficiency of PSII in RAF plants and the most significant differences between RAD and RAF plants were evident after 9–15 h of rehydration. The lower photochemical activity of PSII in RAD plants may be due to their lower water content and higher amount of closed PSII reaction centers. The studies on *R. serbica* showed that the intrinsic PSII efficiency remained at very low levels even when RWC recovered to 36% (Degl'Innocenti *et al.* 2008). After that, a slight increase was observed for 50% RWC, but CO_2 fixation rate did not restore at this time point of rehydration. Our previous studies also revealed that during rehydration the CO_2 assimilation slowly increased, but reached positive values after 72 h of rehydration, when the water content recovered to the values of controls (Péli *et al.* 2012). In agreement with lower photochemical activity of PSII, the excitation pressure on open PSII reaction centers was significantly higher in RAD compared to RAF *H. rhodopensis* plants during the first 9–15 h after rewatering. Thus, it is obvious that the early stage of rehydration is potentially harmful for PSII reaction centers, because of low RWC, low electron transport capacity, and high excitation pressure. Under conditions of high excitation pressure, energy balance can be reestablished by diminishing the light-harvesting antenna size or increased energy dissipation as heat with no change in the absolute antenna size (Hüner *et al.* 2003).

It was found that in desiccated state and during the first hours of rehydration, most of the light absorbed in PSII antennae was allocated to thermal energy dissipation, protecting plants against photooxidation when photosynthetic activity was still not recovered. Another mechanism of thermal energy dissipation was proposed to be activated during desiccation of poikilohydric autotrophs (Heber and Shuvalov 2005, Heber *et al.* 2006a). Loss of water during desiccation in combination with the reduction of Q_A appears to change the conformation of PSII reaction centers so as to transform them from energy-conserving to energy-dissipating centers (Heber *et al.* 2006a, Heber 2008). The role of this additional photoprotective mechanism within PSII reaction centers have been reported for several desiccation tolerant mosses, lichens, and ferns (Heber *et al.* 2006a,b, 2007; Heber 2008, Flores-Bavestrello *et al.* 2016). Our results showed that during rehydration, the 'excess' energy, which was neither utilized in photosynthetic electron transport nor thermally dissipated, gradually increased. The 'excess' energy reflects energy quenching processes occurring within PSII reaction center with Q_A in a reduced state, indicating the role of PSII reaction center quenching during the recovery after rehydration.

The results from fluorescence excitation spectra suggest that during rehydration the size of the light harvesting antenna of PSI does not change significantly for RAD and RAF plants. However, different behavior was observed for PSII complexes − for RAF plants, the involvement of Chl *b* in energy supply of PSII in comparison with Chl *a* decreased gradually with the time of rehydration. High excitation pressure at the beginning of rehydration was accompanied by a corresponding increase of proportion of thermal energy dissipation in the antenna ($1 - F_v'/F_m'$), suggesting its protective effect in severely desiccated plants.

The most prominent alterations in energy transfer and interaction between the main photosynthetic pigment protein complexes as evidenced by 77 K fluorescence emission spectra were observed during the first hours (3 and 7 h) of rehydration. The contours of fluorescence spectra of thylakoid membranes isolated from RAD and RAF were not altered in respect to positions of the main peaks but their relative intensities were changed. The ratio of F_{735}/F_{685} increased during the first 3–7 h of rehydration that was valid for both groups of plants, stronger expressed for the 3^{th} hour. The observed increase in F_{735}/F_{685} ratio could occur due to two reasons. First, as a result of desiccation-induced unstacking of thylakoid membranes more energy is transferred to and emitted by the PSI complex (F_{735}). Second, a decrease of the emission from PSII (F_{685}) could be related to a reduction of the population of PSII complexes (Charuvi *et al.* 2015) and/or fluorescence quenching by inactivated complexes of PSII (Hundal *et al.* 1990, Velitchkova and Popova 2005, Popova *et al.* 2019). It had been shown that in thylakoids and PSII particles, inactivation of the donor side of PSII is accompanied by a formation of a P_{685}^+, a quencher of the fluorescence emitted by PSII reaction center (Horton and Ruban 1992, Bruce *et al.* 1997). The suggestion that the elevated F_{735}/F_{685} ratio after 3 h of rehydration is related to quenching of fluorescence is supported by the decrease of the ratio of F_{685}/F_{695}.

Moreover, the faster enhancement in F_v/F_m values compared to Φ_{PSII} at the beginning of rehydration, when CO_2 assimilation was still negative (Péli *et al*. 2012), can create an energy imbalance between the primary and secondary reactions of photosynthesis, which was confirmed by high excitation pressure under these conditions. This, in turn, can potentially result in generation of reactive oxygen species. Indeed, increased activity of superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, and nonspecific peroxidases was detected, indicating that the first few hours of rehydration represent extremely dramatic period regarding oxidative stress (Sgherri *et al*. 2004, Jovanović *et al*. 2011). On the other hand, the elevated contents of phenols during the first phase of rehydration suggest that they also might have a role in scavenging of ROS (Sgherri *et al*. 2004). The importance of the first few hours of rehydration for plants recovery was also shown by studies of Bernacchia *et al*. (1996) during the rehydration process of dried *Craterostigma plantagineum* plants. It was found that the dehydration-specific gene products disappeared during an early phase of rehydration (within 6 h after the contact with water), a small number of rehydration-specific transcripts was synthesized around 12 to 15 h after the onset of rewatering and hydration-related gene products appeared concomitantly.

Following rehydration, PSI activity recovered faster compared to PSII and in contrast to PSII, its activity was higher in RAD compared to RAF plants. Another feature upon recovery of the activity of this photosystem is the restoration of maximum activity after 15 and 24 h of rehydration of RAF and RAD plants, respectively, when PSII activity significantly increased. In fact, our previous results showed the higher PSI activity in moderately dehydrated *H. rhodopensis* plants compared to well-hydrated plants (Georgieva *et al*. 2005). In recent years, a number of alternative pathways for photosynthetic electron transport, including PSI-dependent cyclic electron flow, the chlororespiratory pathway mediated by a chloroplastic NAD(P)H dehydrogenase that uses stromal NAD(P)H for the nonphotochemical reduction of PQ to PQH_2 and the terminal plastoquinol oxidase (PTOX)-mediated electron flow to O_2 have been heavily implicated to play a critical role in protecting the photosynthetic apparatus against various environmental stresses (Savitch *et al*. 2010, McDonald *et al*. 2011, Ivanov *et al*. 2012). In fact, the enhancement of PSI-dependent cyclic electron flow has been shown to improve the recovery of severely desiccated *Porphyra yezoensis* (Gao and Wang 2012).

The results of our study indicate that the photochemical activity of *H. rhodopensis* regain satisfactory stability after 24 h of rehydration and the differences in photochemical activity of RAD and RAF plants significantly decreased. The quantum efficiency of electron transport trough PSII was more than 70% of the control level, PSI activity was restored, the excitation pressure was significantly reduced and the proportion of thermal energy dissipation in the antenna was close to the fully rehydrated plants. These data are in agreement with our previous study on subcellular organization of mesophyll cells showing that after 1 d of rehydration the inner vacuoles seemed to enlarge, the organelles started to return to their normal position and by the end of the first day of rehydration, the plastids regained their half-lens shape at the plasmalemma (Georgieva *et al*. 2017b).

In summary, it could be concluded that the early stage of rehydration of desiccated to air-dry state *Haberlea rhodopensis* plants is potentially harmful and plants need adequate and effective protection during this period. The most significant differences in the recovery process of photosynthetic activity of RAD and RAF plants were observed after 9–15 h of rehydration. PSI activity recovered more rapidly and PSII activity more slowly in early hours of rehydration of RAD compared to RAF plants. The high thermal energy dissipation in dry plants and during the first hours of their rehydration protect them from photooxidation and the role of PSII reaction center quenching during the recovery period was suggested.

References

Andrizhiyevskaya E.G., Chojnicka A., Bautista J.A. *et al*.: Origin of the F685 and F695 fluorescence in Photosystem II. – Photosynth. Res. **84**: 173-180, 2005.

Benina M., Obata T., Mehterov N. *et al*.: Comparative metabolic profiling of *Haberlea rhodopensis*, *Thellungiella halophyla*, and *Arabidopsis thaliana* exposed to low temperature. – Front. Plant Sci. **4**: 499, 2013.

Bernacchia G., Salamini F., Bartels D.: Molecular characterization of the rehydration process in the resurrection plant *Craterostigma plantagineum*. – Plant Physiol. **111**: 1043-1050, 1996.

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

Bewley J.D., Oliver M.J.: Desiccation tolerance in vegetative plant tissues and seeds: protein synthesis in relation to desiccation and a potential role for protection and repair mechanisms. – In: Somero G.N., Osmond C.B., Bolis C.L. (ed.): Water and Life. Pp. 141-160. Springer, Berlin-Heidelberg 1992.

Bruce D., Samson G., Carpenter C.: The origins of non-photochemical quenching of chlorophyll in photosynthesis. Direct quenching by $P680^{(+)}$ in photosystem II enriched membranes at low pH. – Biochemistry-US **36**: 749-755, 1997.

Charuvi D., Nevo R., Shimoni E. *et al*.: Photoprotection conferred by changes in photosynthetic protein levels and organization during dehydration of a homoiochlorophyllous resurrection plant. – Plant Physiol. **167**: 1554-1565, 2015.

Cooper K., Farrant J.M.: Recovery of the resurrection plant *Craterostigma wilmsii* from desiccation: protection versus repair. – J. Exp. Bot. **53**: 1805-1813, 2002.

Daskalova E., Dontcheva S., Yahoubian G. *et al*.: A strategy for conservation and investigation of the protected resurrection plant *Haberlea rhodopensis* Friv. – BioRisk **6**: 41-60, 2011.

Daskalova E., Dontcheva S., Yahubyan G. *et al*.: Ecological characteristics and conservation of the protected resurrection species *Haberlea rhodopensis* Friv. as *in vitro* plants through a modified micropropagation system. – Biotechnol. Biotec. Eq. **24**: 213-217, 2010.

Degl'Innocenti E., Guidi L., Stevanovic B., Navari F.: CO_2 fixation and chlorophyll a fluorescence in leaves of *Ramonda serbica* during a dehydration-rehydration cycle. – J. Plant Physiol. **165**: 723-733, 2008.

Demmig-Adams B., Adams III W.W., Barker D.H. *et al.*: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Deng X., Hu Z.A., Wang H.X. *et al*.: 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, 2003.

Djilianov D., Ivanov S., Moyankova D. *et al*.: Sugar ratios, glutathione redox status and phenols in the resurrection species *Haberlea rhodopensis* and the closely related non-resurrection species *Chirita eberhardtii*. – Plant Biol. **13**: 767-776, 2011.

Farrant J.M.: Mechanisms of desiccation tolerance in angiosperm resurrection plants. – In: Jenks M., Wood A. (ed.): Plant Desiccation Tolerance. Pp. 51-90. Blackwell Publishing, Wallingford 2007.

Farrant J.M., Brandt W.F., Lindsey G.G.: An overview of mechanisms of desiccation tolerance in selected angiosperm resurrection plants. – Plant Stress **1**: 72-84, 2007.

Farrant J.M., Cooper K., Kruger L.A., Sherwin H.W.: The effect of drying rate on the survival of three desiccation-tolerant angiosperm species. – Ann. Bot.-London **84**: 371-379, 1999.

Fernández-Marín B., Nadal M., Gago J. *et al*.: Born to revive: molecular and physiological mechanisms of double tolerance in a paleotropical and resurrection plant. – New Phytol. **226**: 741-759, 2020.

Fernández-Marín B., Neuner G., Kuprian E. *et al*.: First evidence of freezing tolerance in a resurrection plant: insights into molecular mobility and zeaxanthin synthesis in the dark. – Physiol. Plantarum **163**: 472-489, 2018.

Flores-Bavestrello A., Król M., Ivanov A.G. *et al*.: Two Hymenophyllaceae species from contrasting natural environments exhibit a homoiochlorophyllous strategy in response to desiccation stress. – J. Plant Physiol. **191**: 82-94, 2016.

Gaff D.F., Oliver M.: The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. – Funct. Plant Biol. **40**: 315, 2013.

Gao S., Wang G.: The enhancement of cyclic electron flow around photosystem I improves the recovery of severely desiccated *Porphyra yezoensis* (Bangiales, Rhodophyta). – J. Exp. Bot. **63**: 4349-4358, 2012.

Gashi B., Babani F., Kongjika E.: Chlorophyll fluorescence imaging of photosynthetic activity and pigment contents of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae* during dehydration and rehydration. – Physiol. Mol. Biol. Pla. **19**: 333-341, 2013.

Gashi B., Kongjika E., Osmani M., Luma V.: Activity of δ-aminolevulinic acid dehydratase at *Ramonda nathaliae* and *Ramonda serbica* plants during dehydration and rehydration. – Biol. Futur. **70**: 210-217, 2019.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.

Georgieva K., Dagnon S., Gesheva E. *et al*.: Antioxident defense during desiccation of the resurrection plant *Haberlea rhodopensis*. – Plant Physiol. Bioch. **114**: 51-59, 2017a.

Georgieva K., Maslenkova L., Peeva V. *et al*.: 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., Mihailova G.: Drought tolerance of photosynthesis. – In: Pessarakli M. (ed.): Handbook of Photosynthesis. 3rd Edition. Pp. 683-695. CRC Press, Boca Raton 2016.

Georgieva K., Mihailova G., Gigova L. *et al*.: Freezing tolerance of resurrection plant *Haberlea rhodopensis*.– In: FEBS Advanced Courses – Resurrection Plants: Hope for Crop Drought Tolerance (ReHOPE) (Abstract Book). Pp. 13. 20–22 September, Plovdiv, Bulgaria 2018. https://rehope2018.febsevents.org/

Georgieva K., Rapparini F., Bertazza G. *et al*.: Alterations in the sugar metabolism and in the vacuolar system of mesophyll cells contribute to the desiccation tolerance of *Haberlea rhodopensis* ecotypes. – Protoplasma **254**: 193-201, 2017b.

Georgieva K., Röding A., Büchel C.: Changes in some thylakoid membrane proteins and pigments upon desiccation of the resurrection plant *Haberlea rhodopensis*. – J. Plant Physiol. **166**: 1520-1528, 2009.

Georgieva K., Szigeti Z., Sarvari E. *et al*.: Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during dehydration and rehydration. – Planta **225**: 955-964, 2007.

Giarola V., Bartels D.: What can we learn from the transcriptome of the resurrection plant *Craterostigma plantagineum*? – Planta **242**: 427-434, 2015.

Giarola V., Hou Q., Bartels D.: Angiosperm plant desiccation tolerance: hints from transcriptomics and genome sequencing. – Trends Plant Sci. **22**: 705-717, 2017.

Heber U.: Photoprotection of green plants: a mechanism of ultra-fast thermal energy dissipation in desiccated lichens. – Planta **228**: 641-650, 2008.

Heber U., Azarkovich M., Shuvalov V.: Activation of mechanisms of photoprotection by desiccation and by light: poikilohydric photoautotrophs. – J. Exp. Bot. **58**: 2745-2759, 2007.

Heber U., Bilger W., Shuvalov V.A.: Thermal energy dissipation in reaction centres and in the antenna of photosystem II protects desiccated poikilohydric mosses against photo-oxidation. – J. Exp. Bot. **57**: 2993-3006, 2006b.

Heber U., Lange O.L., Shuvalov V.A.: Conservation and dissipation of light energy as complementary processes: homoiohydric and poikilohydric autotrophs. – J. Exp. Bot. **57**: 1211-1223, 2006a.

Heber U., Shuvalov V.A.: Photochemical reactions of chlorophyll in dehydrated photosystem II: two chlorophyll forms (680 and 700 nm). – Photosynth. Res. **84**: 85-91, 2005.

Horton P., Ruban A.: Regulation of Photosystem II. – Photosynth. Res. **34**: 375-385, 1992.

Huang W., Yang S.J., Zhang S.B. *et al*.: Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress. – Planta **235**: 819-828, 2012.

Hundal T., Virgin I., Styring S., Andersson B.: Changes in the organization of photosystem II following light-induced D1-protein degradation. – BBA-Bioenergetics **1017**: 235-241, 1990.

Hüner N.P., Öquist G., Melis A.: Photostasis in plants, green algae and cyanobacteria: the role of light harvesting antenna complexes. – In: Green B.R., Parson W.W. (ed.): Light-Harvesting Antennas in Photosynthesis. Advances in Photosynthesis and Respiration. Vol. 13. Pp. 401-421. Springer, Dordrecht 2003.

Ivanov A.G., Rosso D., Savitch L.V. *et al*.: Implications of alternative electron sinks in increased resistance of PSII and PSI photochemistry to high light stress in cold acclimated *Arabidopsis thaliana*. – Photosynth. Res. **113**: 191-206, 2012.

Jovanović Ž., Rakić T., Stevanović B., Radović S.: Characterization of oxidative and antioxidative events during dehydration and rehydration of resurrection plant *Ramonda nathaliae*. – Plant Growth Regul. **64**: 231-240, 2011.

Kappen L.: [The influence of water content on the resistance of plants to high and low temperatures, examined on leaves of some ferns and *Ramonda myconi*.] – Flora **156**: 427-445, 1966. https://doi.org/10.1016/S0367-1836(17)30278-1 [In German]

Krause G.H., Weis E.: Chlorophyll fluorescence and photosynthesis. The basics. – Annu. Rev. Plant Phys. **42**: 313-349, 1991.

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.

Lichtenthaler H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.

Liu J., Moyankova D., Djilianov D., Deng X.: Common and specific mechanisms of desiccation tolerance in two Gesneriaceae resurrection plants. Multiomics evidences. – Front. Plant Sci. **10**: 1067, 2019.

McDonald A.E., Ivanov A.G., Bode R. *et al.*: Flexibility in photosynthetic electron transport: the physiological role of plastoquinol terminal oxidase (PTOX). – BBA-Bioenergetics **1807**: 954-967, 2011.

Morse M., Rafudeen M.S., Farrant J.M.: An overview of the current understanding of desiccation tolerance in the vegetative tissues of higher plants. – In: Turkan I. (ed.): Advances in Botanical Research. Vol. 57. Pp. 319-347. Elsevier, Amsterdam 2011.

Oliver M.J., Tuba Z., Mishler B.D.: The evolution of vegetative desiccation tolerance in land plants. – Plant Ecol. **151**: 85-100, 2000.

Peeva V., Cornic G.: Leaf photosynthesis of *Haberlea rhodopensis* before and during drought. – Environ. Exp. Bot. **65**: 310-318, 2009.

Péli E.R., Mihailova G., Petkova S. *et al.*: Differences in physiological adaptation of *Haberlea rhodopensis* Friv. leaves and roots during dehydration-rehydration cycle. – Acta Physiol. Plant. **34**: 947-955, 2012.

Petrova G., Moyankova D., Nishii K. *et al.*: The European paleoendemic *Haberlea rhodopensis* (Gesneriaceae) has an Oligocene origin and a Pleistocene diversification and occurs in a long-persisting refugial area in Southeastern Europe. – Int. J. Plant Sci. **176**: 499-514, 2015.

Popova A.V., Dobrev K, Velitchkova M., Ivanov A.G.: Differential temperature effects on dissipation of excess light energy and energy partitioning in *lut2* mutant of *Arabodopsis thaliana* under photoinhibitory conditions. – Photosynth. Res. **139**: 367-385, 2019.

Rakić T., Lazarević M., Jovanović Ž.S. *et al.*: Resurrection plants of the genus *Ramonda*: prospective survival strategies – Unlock further capacity of adaptation, or embark on the path of evolution? – Front. Plant Sci. **4**: 550, 2014.

Savitch L.V., Ivanov A.G., Krol M. *et al.*: Regulation of energy partitioning and alternative electron transport pathways during cold acclimation of lodgepole pine is oxygen dependent. – Plant Cell Physiol. **51**: 1555-1570, 2010.

Sgherri C., Stevanović B., Navari-Izzo F.: Role of phenolic in the antioxidative status of the resurrection plant *Ramonda serbica* during dehydration and rehydration. – Physiol. Plantarum **112**: 478-485, 2004.

Tan T., Sun Y., Luo S. *et al.*: Efficient modulation of photosynthetic apparatus confers desiccation tolerance in the resurrection plant *Boea hygrometrica*. – Plant Cell Physiol. **58**: 1976-1990, 2017.

van Kooten O., Snel J.F.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Velitchkova M., Popova A.: High light-induced changes of 77 K fluorescence emission of pea thylakoid membranes with altered membrane fluidity. – Bioelectrochemistry **67**: 81-90, 2005.

Vertucci C.W., Farrant J.M.: Acquisition and loss of desiccation tolerance. – In: Kigel J., Galili G. (ed.): Seed Development and Germination. Pp. 237-271. Marcel Dekker, New York 1995.

Živković T., Quartacci M.F., Stevanović B. *et al.*: Low-molecular weight substances in the poikilohydric plant *Ramonda serbica* during dehydration and rehydration. – Plant Sci. **168**: 105-111, 2005.