B4-01

Plant Growth Regul (2012) 67:121–132
DOI 10.1007/s10725-012-9669-3

ORIGINAL PAPER

Response of sun- and shade-adapted plants of *Haberlea rhodopensis* to desiccation

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Received: 8 June 2011 / Accepted: 7 February 2012 / Published online: 18 February 2012

Abstract The differences in some morphological and physiological characteristics of sun- and shade-adapted *Haberlea rhodopensis* plants were compared. Changes in the photosynthetic activity, electrolyte leakage from leaf tissues, malondialdehyde content (MDA) and leaf anatomy were studied at different degrees of desiccation as well as after rehydration of plants. The MDA content in well-watered sun *Haberlea* plants was higher compared to shade plants suggesting higher lipid peroxidation, which is commonly regarded as an indicator of oxidative stress, but desiccation of plants at high light did not cause additional oxidative damage as judged by the unaffected MDA content. The electrolyte leakage from dried leaves (8% RWC) from both shade and sun plants increased fourfold indicating similar membrane damage. However, the recovery after rehydration showed that this damage was reversible. Well-watered sun plants had higher photosynthetic activity probably due to the larger thickness of the mesophyll layer in such plants. On the other hand, desiccation at high light reduced CO_2 assimilation which was in accordance with the stronger reduction of stomatal conductance. Stomata were visible only on the abaxial side of sun leaves having also higher abundance of non-glandular trichomes. Increased trichomes density and epicuticular waxes and filaments upon desiccation could help plants to increase reflection, reduce net radiation income, slow down the rate of water loss and survive adverse conditions.

Keywords Desiccation · Leaf structure · Photosynthesis · Resurrection plant

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Introduction

Resurrection plants have the unique ability to survive desiccation to the air-dry state. Upon watering, the plants rapidly revive and restore to their former state. The desiccation tolerance can depend on different strategies carried out by the plants to survive the severe water loss (Proctor and Pence 2002). Plant cells can either constantly maintain the processes and components necessary to protect cells, rendering desiccation tolerance a constitutive trait, or these can be induced when dehydrating conditions are encountered. Similarly the processes and activities associated with repair could be constitutive or induced following rehydration (Oliver et al. 2005). In lower orders such as bryophytes, dehydration is rapid and plants rely mostly on constitutive mechanisms of tolerance and institution of effective repair mechanisms (Proctor and Smirnoff 2000; Proctor et al. 2007). In higher plants, dehydration is slower and most of the protection is laid down during dehydration (Farrant et al. 2007). If dehydration is too rapid at this stage, effective protection is not accrued and recovery is compromised (Oliver 1996; Cooper and Farrant 2002). For this reason, apart from the physiological and metabolic mechanisms, also the anatomy, morphology and ultrastructural characteristics of the leaf, useful in slowing down the water loss rate, may be particularly significant in enabling the cells to bear extreme dehydration in their natural environmental conditions (Vecchia et al. 1998).

The photosynthetic apparatus is very sensitive and liable to injury, and needs to be maintained or quickly repaired upon rehydration (Farrant 2000; Ramanjulu and Bartels 2002; Farrant et al. 2007). Water relations in plants are regulated largely via the opening and closure of stomata. Water deficit leads to a closure of stomata and at the same

B4-02

Journal of Plant Physiology 171 (2014) 1591–1600

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Comparison of thylakoid structure and organization in sun and shade *Haberlea rhodopensis* populations under desiccation and rehydration

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ARTICLE INFO

Article history:
Received 29 May 2014
Received in revised form 29 July 2014
Accepted 30 July 2014
Available online 12 August 2014

Keywords:
Blue-Native PAGE
Chlorophyll–protein complexes
Electron microscopy
Steady-state 77 K fluorescence
Resurrection plant

ABSTRACT

The resurrection plant, *Haberlea rhodopensis* can survive nearly total desiccation only in its usual low irradiation environment. However, populations with similar capacity to recover were discovered recently in several sunny habitats. To reveal what kind of morphological, structural and thylakoid-level alterations play a role in the acclimation of this low-light adapted species to high-light environment and how do they contribute to the desiccation tolerance mechanisms, the structure of the photosynthetic apparatus, the most sensitive component of the chlorophyll-retaining resurrection plants, was analyzed by transmission electron microscopy, steady state low-temperature fluorescence and two-dimensional Blue-Native/SDS PAGE under desiccation and rehydration.

In contrast to the great differences in the morphology of plants, the ultrastructure and the organization of thylakoids were surprisingly similar in well-hydrated shade and sun populations. A high ratio of photosystem (PS)I binding light harvesting complex (LHC)II, important in low- and fluctuating light environment, was characteristic to both shade and sun plant, and the ratios of the main chlorophyll–protein complexes were also similar. The intensive protective mechanisms, such as shading by steep leaf angle and accumulation of protective substances, probably reduced the light intensity at the chloroplast level. The significantly increased ratio of monomer to oligomer antennae in well-hydrated sun plants may be connected with the temporary high light exposure of chloroplasts.

During desiccation, LHCII was removed from PSI and part of PSII supercomplexes disassembled with some loss of PSII core and LHCII. The different reorganization of antennae, possibly connected with different quenching mechanisms, involved an increased amount of monomers in shade plants but unchanged proportion of oligomers in sun plants. Desiccation-induced responses were more pronounced in sun plants which also had a greater capacity to recover due to their stress-acclimated attitude.

Introduction

Environmental stresses, including the world-wide prevalent drought, deeply influence plant productivity. However, plants, being sessile, evolved various mechanisms for acclimation to environmental challenges. Due to the significance of photosynthesis in plant life, acclimation mechanisms of the photosynthetic apparatus are processes of prime importance. Concerning the chlorophyll–protein (Chl–protein) complexes, heart of the photosynthetic apparatus, plasticity involves alterations in their interactions on a short-term and in their stoichiometry on a

Abbreviations: BN, Blue-Native; Chl, chlorophyll; DGDG, digalactosyl-diacylglycerol; Lhc, light-harvesting complexes; LHCII, trimer light-harvesting complex; MGDG, monogalactosyl-diacylglycerol; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; RWC, relative water content; SDS, sodium dodecyl sulphate.

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http://dx.doi.org/10.1016/j.jplph.2014.07.015

B4-03

Environmental and Experimental Botany 113 (2015) 67–79

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Growth irradiance affects the photoprotective mechanisms of the resurrection angiosperm *Haberlea rhodopensis* Friv. in response to desiccation and rehydration at morphological, physiological and biochemical levels

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ARTICLE INFO

Article history:
Received 9 September 2014
Received in revised form 23 January 2015
Accepted 27 January 2015
Available online 31 January 2015

Keywords:
Desiccation tolerance
Energy partitioning
Haberlea rhodopensis
Growth irradiance
Photoprotection
Xanthophylls
Carotenoids

ABSTRACT

In the present study, we examined the photoprotective mechanisms of the homoiochlorophyllous desiccation-tolerant (DT) *Haberlea rhodopensis* Friv. (Gesneriaceae) during the desiccation–rehydration cycle in its natural understory shaded and sunny habitats within drought prone regions. The integration of classical analysis of chlorophyll fluorescence with a detailed analysis of energy partitioning showed a re-adjustment in the function and extent of the different components of energy management in photosystem II (PSII) depending on the degree of dehydration. At mild dehydration, the non-photochemical quenching by active centres (NPQ) played a relevant role in preventing photoinhibition, while under the photoinhibitory conditions occurring when dehydration was severe, the light-dependent quenching by inactive centres provided strong photoprotection. These analyses of the photosynthetic and PSII functionality together with measurements of carotenoid changes showed that the photoprotective mechanisms of this resurrection species were also affected by growth irradiance. Plants growing in understory shaded habitats were mainly protected against dehydration by the mechanism of thermal re-emission by inactive PSII reaction centres together with the relevant contribution of an operative xanthophyll-cycle involved in a quenching process triggered by dehydration itself. Conversely, plants growing in sunny open environments relied more on the quenching mechanism in the light-harvesting antennae. One additional role of carotenoids, in particular of β-carotene, in dehydration tolerance of *H. rhodopensis* may well be quenching of ROS, a mechanism that could occur in homoiochlorophyllous resurrection plants as a consequence of the retained chlorophylls and photosynthetic apparatus, especially when plants grow in open sunny habitats.

Abbreviations: A, CO_2 assimilation; A, antheraxanthin; DT, desiccation-tolerant; g_s, stomatal conductance; LNU, proportion of light not used for photochemistry; F, actual level of fluorescence; F_o and F_m, minimum and maximum fluorescence yield for open and closed PSII reaction centres, respectively, for a dark-adapted leaf; F'_m, maximum fluorescence for a light-adapted leaf; $(F'_m-F)/F'_m$, actual PSII efficiency during illumination; F_v, variable fluorescence (F_m-F_o); F_v/F_m, maximum PSII efficiency at open centres in dark-adapted leaf; F'_v/F'_m, maximum PSII efficiency at open centres under illumination; 1-(F'_v/F'_m), relative proportion of the energy absorbed and dissipated as heat in the PSII antennae; F_v/F_{mM}, quantum yield efficiency of well hydrated plants, where F_{mM} is the maximum florescence; 1-qP, estimated degree of closure of PSII; NPQ, non-photochemical fluorescence quenching; PSII, photosystem II; V, violaxanthin'; VDE, violaxanthin de-epoxidase; Z, zeaxanthin; ZEP, zeaxanthin epoxidase; WUE, water use efficiency; Φ_{PSII}, quantum efficiency of photochemical transport used for photosynthesis; $\Phi_{f,D}$, combined quantum efficiency of fluorescence and constitutive; Φ_{NPQ}, quantum yield of light-dependent and ΔpH- and xanthophyll-mediated regulated thermal dissipation in active PSIIs; Φ_{NF}, quantum yield of thermal dissipation in photoinactivated.

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http://dx.doi.org/10.1016/j.envexpbot.2015.01.007
0098-8472/

Plant Physiology and Biochemistry 114 (2017) 51–59

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Antioxidant defense during desiccation of the resurrection plant *Haberlea rhodopensis*

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ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
24 February 2017
Accepted 27 February 2017
Available online 28 February 2017

Keywords:
Antioxidant activity
Ascorbate-glutathione cycle
Desiccation tolerance
Polyphenols
Resurrection plants

ABSTRACT

Maintaining a strong antioxidant system is essential for preventing drought-induced oxidative stress. Thus, in the present study we investigated the role of some non-enzymic and enzymic antioxidants in desiccation tolerance of *Haberlea rhodopensis*. The effects of high light upon desiccation on antioxidant capacity was estimated by comparing the response of shade and sun plants. The significant enhancement of the antioxidant capacity at 8% RWC corresponded to an enormous increase in flavonoid content. The important role of ascorbate-glutathione cycle in overcoming oxidative stress during drying of *H. rhodopensis* was established. The antioxidant capacity increased upon dehydration of both shade and sun plants but some differences in non-enzymatic and enzymatic antioxidants were observed.

Investigations on the role of polyphenols in desiccation tolerance are scarce. In the present study the polyphenol profiles (fingerprints) of the resurrection plant *Haberlea rhodopensis*, including all components of the complex are obtained for the first time. It was clarified that the polyphenol complex of *H. rhodopensis* includes only two types of glycosides - phenylethanoid glucosides and hispidulin 8-C-glucosides. Upon desiccation the polyphenol content increase and the main role of phenylethanoid glucosides in the protection of *H. rhodopensis* was revealed.

1. Introduction

Drought is one of the major environmental factors that inhibits many metabolic processes and constrains plant growth and crop productivity. The ongoing global warming and current climate changes enlarge the land areas where plants experience water deficit. That is why it is very important to understand the mechanisms that plants have evolved to cope with drought stress. Desiccation-tolerant (DT) or resurrection plants are excellent model systems for the study the cellular and molecular mechanisms underlying desiccation tolerance.

Oxidative stress is one of the most deleterious consequences of water deprivation. When CO_2 assimilation is decreased at low water content, the electron transport becomes strongly reduced and electron transfer to oxygen increases, producing reactive oxygen species (ROS) (Lawlor and Cornic, 2002). The formation of ROS results in damage to essential cellular components such as nucleic acids, polysaccharides, proteins and membrane lipids (Mundree et al., 2002). Thus ROS play a crucial role in causing cellular damage under drought stress. In addition, the presence of high light intensities during dehydration can be extremely damaging to photosynthetically active tissues (Sherwin and Farrant, 1998). The capacity of the antioxidative defence system determines the fate of the cell and whether the cell continues to function or suffers photooxidation (Ramanjulu and Bartels, 2002). When responses to desiccation are compared, both DT and sensitive species show stimulation of antioxidant activity at one or another point in the drying–rehydration cycle but it generally appears that oxidative damage in the DT species is well controlled whereas the sensitive species show clear signs of oxidative damage (Proctor and Tuba, 2002).

Resurrection plants are protected against the potential damage of desiccation-induced ROS production by: minimizing the

Abbreviations: DT, desiccation tolerance; HAG, hispidulin 8-C-(6-O-acetyl- β-glucopyranoside); HASG, hispidulin 8-C-(6-O-acetyl-2-O-syringoyl- β-glucopyranoside); HSG, hispidulin 8-C-(2-O-syringoyl-β-glucopyranoside); HDT, homoiochlorophyllous desiccation-tolerant plants; Myc, myconoside; Pauc, paucifloside.

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http://dx.doi.org/10.1016/j.plaphy.2017.02.021
0981-9428/

Г7-01

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 65, No 5, 2012

BIOLOGIE

Physiologie des plantes

PHOTOCHEMICAL EFFICIENCY OF PHOTOSYSTEM II DURING DESICCATION OF SHADE- AND SUN-ADAPTED PLANTS OF *HABERLEA RHODOPENSIS*

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(*Submitted by Academician K. Kumanov on November 15, 2011*)

Abstract

The changes in the photochemical activity of PSII and energy dissipation during dehydration of shade and sun *Haberlea rhodopensis* plants from different habitats were examined. The photochemical activity of sun plants was higher compared to shade plants during desiccation to relative water content (RWC) of 50%. Further water loss sharply decreased the photochemical activity. The increase in the thermal energy dissipation, expressed as non-photochemical quenching, had a major role in preventing photoinhibition upon desiccation up to 50% RWC and 20% RWC of shade and sun plants respectively, while at extremely low RWC other mechanisms may become important to avoid photodamage.

Key words: resurrection plants, photochemical activity, non-photochemical quenching, photoinhibition

Introduction. Under field conditions plants are subjected to multiple stresses, such as drought and unfavourable temperature concomitant with high irradiance, that threaten their growth and survival. Any stress that reduces the rate of photosynthetic electron transport and the light-saturated rate of photosynthesis enhances the amount of excess excitation energy [1]. Exposure to excessive energy, arising either from high irradiance incident on the leaf or as a consequence of drought stress, is potentially harmful and can induce photoinhibition of photosystem II (PSII) reaction centres and enhanced production of reactive

This work was supported by the National Science Fund of the Bulgarian Ministry of Education, Youth and Science under Project DO02-208/2008.

Characterization of Energy Transfer Processes and Flash Oxygen Yields of Thylakoid Membranes Isolated from Resurrection Plant *Haberlea Rhodopensis* Subjected to Different Extent of Desiccation

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Abstract: The resurrection plants are unique with their extra desiccation tolerance. The physico-chemical properties of photosynthetic apparatus are of crucial importance for survival of plants upon water stress. In present work the effect of different extent of desiccation on the energy transfer properties and oxygen evolving capacity of isolated thylakoid membranes from resurrection plant *Haberlea Rhodopensis* are investigated. The plants from different habitats in Bulgaria are compared. Energy distribution and spillover between both photosystems are studied by means of 77 K chlorophyll fluorescence. The dependence of fluorescence ratio F735/F685 on the degree of desiccation of plants is also followed. Functionality of PSII and especially of oxygen-evolving apparatus under water deficit is estimated by flash oxygen yields and initial oxygen burst of thylakoid membranes isolated from plants desiccated up to 50% and 8% relative water content (RWC). Population of S_i states as well as the misses and the double hits are calculated according non-cooperative Kok's model and compared for plants from different habitats and different RWC. The results are discussed in terms of involvement of "fast" and "slow" centers from grana and stroma regions in oxygen evolution and alteration of their contribution as a result of desiccation.

Keywords: Desiccation; Energy transfer; Flash oxygen yields; *Haberlea Rhodopensi;,* Resurrection plants

Introduction

One of the more severe environmental factors that damages higher plants growth and productivity is drought. Most of the plants could not survive under desiccation up to air-dried state. A limited number of plants representing so called resurrection plants exhibit a remarkable tolerance to water deficit and under rehydration restore their functions (Moore *et al.*, 2009). The most sensitive part of photosynthetic apparatus to stress factor is oxygen evolving complex and photosystem II (Canaani *et al.*, 1986; Giardi *et al.*, 1996). On the other hand the effect of desiccation on thylakoid membranes and on granal structure is also of interest in order to understand the mechanism of resurrection plant tolerance to severe desiccation and the possibility to restore after rehydration.

In the present paper we studied the oxygen evolution activity of thylakoid membranes isolated from *Haberlea rhodopensis* plants from different habitats. In order to characterize the energy interaction between both photosystems 77 K fluorescence was studied of thylakoids from control and desiccated plants. Fluorescence emission at 77 K demonstrated the alterations in the overall distribution of excitation energy between PSII and PSI as well as stress-induced changes in energy interaction between pigment-protein complexes (Krause and Weis, 1991). The changes of oxygen-evolving capacity of isolated thylakoid membranes are characterized by flash induced oxygen yields (Kok *et al.*, 1970; Zeinalov, 1982). The changes of fluorescence emission and oxygen yields are discussed in terms of possible involvement of desiccation-induced structural reorganization of thylakoids and lateral rearrangement of pigment-protein complexes of PSI, PSII, LHCI and LHCII.

Effect of Light on the Photosynthetic Activity during Desiccation of the Resurrection Plant *Haberlea Rhodopensis*

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Abstract: The effect of light during desiccation of the resurrection plant *Haberlea rhodopensis* on the photosynthetic activity and some morphological parameters was evaluated using plants growing at low or high irradiance in natural habitat. Chlorophyll content was not only lower in sun plants compared to shade plants, but it declined to a higher extent when desiccation was carried out at high light irradiance. Regardless of lower chlorophyll content in sun plants their photosynthetic activity (P_N) was about 30% higher compared to shade plants. However, during dehydration P_N declined more rapidly in sun plants. The mean leaf thickness of fully hydrated leaves from sun plants was larger when compared with shade plants, which was due to higher thickness of the mesophyll. Following rehydration plants rapidly recovered and P_N was higher by about 70% in sun than in shade plants. The results showed that the sun-exposed *Haberlea* plants exhibited good adaptation to desiccation under high irradiance.

Keywords: Desiccation; Leaf thickness; Photosynthesis; Resurrection plant

Introduction

Haberlea rhodopensis belongs to a small group of angiosperms, referred to as “resurrection plants” because they are capable of tolerating extremes of desiccation. It prefers shaded, northern, chiefly limestone slopes but can be found also on the sun-exposed rocks. 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). However, it was found that these plants were very sensitive to photoinhibition (Georgieva and Maslenkova, 2006). Desiccation of shade-adapted plants at irradiance of 350 μmol m^{-2} s^{-1} PPFD induced irreversible changes in the photosynthetic apparatus, and leaves (except the youngest ones) did not recover after rehydration (Georgieva *et al*., 2008). The aim of the present study was to evaluate the effect of light during desiccation of *Haberlea* using plants growing at low or high irradiance in natural habitat. Changes in the photosynthetic activity and some morphological parameters were studied at different degrees of desiccation as well as after rehydration of plants.

Materials and Methods

Well-hydrated and naturally dried *Haberlea rhodopensis* plants growing on rocks below trees in deep shade (Bachkovo region) or sun exposed but briefly shaded by neighboring trees (Sitovo region) were studied. Adult rosettes of similar size and appearance were selected for the experiments. All measurements were conducted on fully expanded mature leaves from control (90% RWC), moderately (50% RWC), severely dehydrated plants (25% RWC) and dried leaves (8% RWC) as well as after 5 days of rehydration of dry plants. The RWC was determined gravimetrically by weighing *Haberlea* leaves before and after oven drying at 80 °C to a constant mass and expressed as the percentage of water content in

Г7-04

Journal of Photochemistry and Photobiology B: Biology 130 (2014) 217–225

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effects of habitat light conditions on the excitation quenching pathways in desiccating *Haberlea rhodopensis* leaves: An Intelligent FluoroSensor study

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ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 6 November 2013
Accepted 14 November 2013
Available online 26 November 2013

Keywords:
Haberlea rhodopensis
Excitation energy allocation
Intelligent FluoroSensor
Far-red fluorescence
Resurrection plant

ABSTRACT

Resurrection plants can survive dehydration to air-dry state, thus they are excellent models of understanding drought and dehydration tolerance mechanisms. *Haberlea rhodopensis*, a chlorophyll-retaining resurrection plant, can survive desiccation to relative water content below 10%. Leaves, detached from plants of sun and shade habitats, were moderately (~50%) dehydrated in darkness. During desiccation, chlorophyll *a* fluorescence was detected by the recently innovated wireless Intelligent FluoroSensor (IFS) chlorophyll fluorometer, working with three different detectors: a pulse-amplitude-modulated (PAM) broadband channel and two channels to measure non-modulated red and far-red fluorescence. No change in area-based chlorophyll content of leaves was observed. The maximal quantum efficiency of photosystem II decreased gradually in both shade and sun leaves. Shade leaves could not increase antennae-based quenching, thus inactivated photosystem II took part in quenching of excess irradiation. Sun leaves seemed to be pre-adapted to quench excess light as they established an intensive increase in antennae-based non-photochemical quenching parallel to desiccation. The higher far-red to red antennae-based quenching may sign light-harvesting complex reorganization. Thus, compared to PAM, IFS chlorophyll fluorometer has additional benefits including (i) parallel estimation of changes in the Chl content and (ii) prediction of underlying processes of excitation energy quenching.

1. Introduction

Desiccation tolerance is widespread among bryophytes and lichens, but rare among angiosperms [1]. Because of their larger size and controlled transpiration, vascular plants perform a more stable water regime than lower plants, thus higher plants survive drought mainly by decreasing the water loss. Some higher plants, however, are able to tolerate desiccation of their tissues. Resurrection plants are unique in their ability to survive dehydration until a quiescent stage is achieved. Upon watering, desiccated plants rapidly rehydrate and restore to their former state. Thus, they are excellent model systems of studying the mechanisms how plants can survive drought and dehydration [2]. Protection against desiccation damage includes the production of non-reducing di- and oligosaccharides, specific proteins such as the late embryogenesis abundant proteins, dehydrins, and heat shock proteins, as well as changes in lipid composition [3–5]. Nevertheless, it is known that one specific mechanism does not confer tolerance on its own rather it is the interplay of several mechanisms which is essential. The protective mechanisms against desiccation in angiosperms have not been fully understood yet, and vary among species [6].

Haberlea rhodopensis Friv. is a perennial, herbaceous, chlorophyll-retaining resurrection flowering plant. It is a pre-glacial tertiary relict species endemic in the mountains of Bulgaria (Balkan, Rhodope). It colonises calcareous rock surfaces on the natural habitat. *H. rodopensis* belongs to the tropical–subtropical family *Gesneriaceae*, thus a close relative of the ornamental plant, African violet. It prefers the shady, northward slopes and high humidity regions of limestone ridges. Previous investigations have indicated that *H. rhodopensis* leaves as well as whole plants are able to survive desiccation to relative water content (RWC) below 10% in the dark or at low light intensity (about 30 μmol m^{-2} s^{-1}) [7]. However, desiccation at irradiance of 350 μmol m^{-2} s^{-1} induced irreversible changes in the photosynthetic apparatus, and mature leaves did not recover after rehydration [8]. Nevertheless, plants occupying habitats of high light intensity were discovered recently, which were able to recover under the natural high irradiation.

In *H. rhodopensis*, the photosynthetic apparatus is retained during desiccation. Photosynthesis is known to be very sensitive

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1011-1344/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2013.11.016

Volume 58(1):11-14, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Antioxidative defence mechanisms contributes to desiccation tolerance in *Haberlea rhodopensis* population naturally exposed to high irradiation

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ABSTRACT Drought induced stress is one of the most important among the environmental challenges. *Haberlea rhodopensis*, a chlorophyll-retaining resurrection plant, can survive desiccation to air-dry stage in its usual low irradiance habitat ("shade" plants). Nevertheless, in the past years, some populations living under high irradiance ("sun" plants) have been also discovered with the same ability to survive dehydration. In order to clarify the adaptation mechanisms to a high irradiation habitat, superoxide dismutase (SOD) activity determined by activity staining on polyacrylamide gels and malondialdehyde (MDA) content of sun and shade plants collected from high and low irradiance environment, respectively, were studied. Desiccation induced a significantly higher induction in SOD activity and thus a smaller increase in the MDA content in sun compared to shade plants. The MDA content and SOD activity was restored in both sun and shade plants after six-day rehydration. Nevertheless, the SOD activity remained higher in rehydrated sun leaves compared to the well-hydrated initial stage. The early enhancement of SOD activity in dehydrating sun plants contributes to the higher stress tolerance of these populations.

Acta Biol Szeged 58(1):11-14 (2014)

KEY WORDS

drought
Haberlea rhodopensis
malondialdehyde
resurrection plant
superoxide dismutase

Environmental challenges deeply influence plant productivity. However, plants evolved mechanisms in order to tolerate various stresses including drought. One of the most amazing set of tolerance mechanisms is evolved by the resurrection higher plants which have a unique ability to survive dehydration to the air-dry state. *Haberlea rhodopensis* Friv. (Gesneriaceae) is a Balkan endemism that belongs to the homoiochlorophyllous (chlorophyll-retaining) resurrection type (Tuba et al. 1998). Most of the *H. rhodopensis* populations grow in deep shadow under natural conditions, and they are very sensitive to high irradiation especially during desiccation (Georgieva and Maslenkova 2006). In fact, desiccation at 350 µmol m^{-2} s^{-1} irradiance induced irreversible damages in the photosynthetic apparatus, and thus mature leaves were not able to recover during rehydration (Georgieva et al. 2008). Nevertheless, recent studies revealed a high ecological plasticity of *H. rhodopensis* in natural habitats (Daskalova et al. 2011). In spite of the majority of *H. rhodopensis* prefers deeply shaded light environment, several populations inhabit rock surfaces exposed to high light irradiance, drought, high and low temperatures. Previous results showed that the membrane integrity of sun plants is protected (Georgieva et al. 2012). They were shown to have a higher ability to dissipate absorbed excitation energy by an antennae-based non-photochemical quenching route, thus have a reduced photosystem II inactivation during desiccation in contrast to shade plants (Solti et al. 2014). It is also known that resurrection plants use antioxidative defence systems to cope with desiccation (Farrant 2000; Dinakar et al. 2012). For a better understanding of the adaptation of sun plant to the high-light habitat, the oxidative damage and antioxidative defence was compared in *H. rhodopensis* sun and shade plants.

Accepted July 30, 2014
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Materials and Methods

Plant material

Experiments were conducted on *Haberlea rhodopensis* Friv. plants growing in Rhodope Mountains, South-West Bulgaria, Orehovo region (N41°52.231; E024°36.171). Plants of various hydration stages were collected on slopes exposed to full sunlight (1500-1700 µmol m^{-2} s^{-1} average summer photosynthetically active radiation [PAR], leaf-level temperature of 30-37 °C and relative air humidity of about 15-30%; 'sun' plants). Sun plants were compared to understory plants growing in shaded habitats (25 µmol m^{-2} s^{-1} average summer PAR, leaf-level temperature of 21-25 °C and a relative humidity of 40-45%; 'shade' plants). Light intensity was measured at the surface of the collected plants by QSPAR Quantum Sensor (Hansatech Ltd., United Kingdom). Leaf temperature and

GENETICS AND PLANT PHYSIOLOGY – 2015, VOLUME 5(1), PP. 74–85
Special Issue – Project BG051PO001-3.3.06-0025 "Support for training and development of young competitive experts in the field of physiology, phytochemistry, genomics, proteomics and biodiversity of eukaryotic organisms"

Available online at http://www.ifrg-bg.com

PHOTOSYNTHETIC CHARACTERISTICS OF THE RESURRECTION PLANT *HABERLEA RHODOPENSIS* FROM TWO HABITATS

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Received: *04 December 2014* Accepted: *23 January 2015*

Summary: The photosynthetic characteristics of *Haberlea rhodopensis* plants from two habitats in Western Rhodope Mountain (in the regions of Trigrad and Wonderful bridges) were studied. Although the plants from both habitats were exposed to sun during part of the day, their leaves were morphologically similar to those of shade plants. The desiccation-induced damages on membrane integrity were characterized by electrolyte leakage and lipid peroxidation. Photochemical activity, energy distribution between the two photosystems and physicochemical properties of thylakoid membranes were estimated by means of chlorophyll fluorescence, 77K fluorescence and particle microelectrophoresis measurements. The mutual organization of PSI-LHCI and PSII-LHCII complexes and spillover between them were similar in thylakoids isolated from well-hydrated plants from Trigrad and Wonderful bridges. Under desiccation up to 8% RWC the F735/F685 ratio at excitation with 472 nm (chl *b*) remained almost unchanged in thylakoid membranes from Wonderful bridges while it decreased considerably in thylakoid membranes from Trigrad. The thylakoid membranes from Wonderful bridges showed a less alteration in energy distribution between both photosystems in response to dehydration. Dehydration induced stronger modifications in thylakoid membranes of plants from Trigrad, expressed by higher electrolyte leakage, lipid peroxidation and membrane surface charge density.

Citation: Mihailova G., M. Velitchkova, V. Doltchinkova, D. Lazarova, K. Georgieva, 2015. Photosynthetic characteristics of the resurrection plant *Haberlea rhodopensis* from two habitats. *Genetics and Plant Physiology*, 5(1): 74–85.

Keywords: *Haberlea rhodopensis*; desiccation; photosynthesis; energy transfer; surface charge density.

Abbreviations: Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; d.c. – direct current; EPM – electrophoretic mobility; ETR – electron transport rate; LHC – light-harvesting complex; NPQ – non-photochemical quenching; PS – photosystem; RWC – relative water content; ζ – zeta potential; σ – surface charge density.

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Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 69, No 1, 2016

BIOLOGIE

Physiologie végétale

DESICCATION INDUCED CHANGES IN PHOTOSYNTHESIS RELATED PROTEINS OF SHADE AND SUN *HABERLEA RHODOPENSIS* PLANTS

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(*Submitted by Academician K. Koumanov on November 13, 2015*)

Abstract

The changes in the content of some proteins involved in the light reactions of photosynthesis were examined during desiccation and after rehydration of shade and sun *H. rhodopensis* plants. Desiccation of plants to air-dry state resulted in a decreased level of the main proteins of both photosystems. The level of PSI reaction centre protein, PsaB was less affected by desiccation compared to the PSII reaction centre protein, PsbA, which is in agreement with the earlier observed higher decline in photochemical activity of PSII compared to PSI. Drought stress reduced the amount of the investigated thylakoid proteins more strongly in shade plants compared to sun plants and the recovery of the latter after rehydration was better.

Key words: desiccation tolerance, thylakoid membrane proteins, resurrection plants

Introduction. Desiccation tolerant or resurrection plants are unique among angiosperms in their ability to survive dehydration to an air-dry state. This is the severest form of water stress, since most protoplasmic water is lost from the cell under these conditions. Upon watering the plants rapidly revive and are

We gratefully acknowledge support from the DAAD (grant number 54392417) and Bulgarian Science Fund (DNTS/Germany 01/0001).

Protoplasma (2017) 254:193–201
DOI 10.1007/s00709-015-0932-0

ORIGINAL ARTICLE

Alterations in the sugar metabolism and in the vacuolar system of mesophyll cells contribute to the desiccation tolerance of *Haberlea rhodopensis* ecotypes

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Received: 22 September 2015 / Accepted: 15 December 2015 / Published online: 23 December 2015

Abstract *Haberlea rhodopensis* belongs to the small group of resurrection plants having the unique ability to survive desiccation to air dry state retaining most of its chlorophyll content and then resume normal function upon rehydration. It prefers the shady valleys and northward facing slopes of limestone ridges in mountain zones with high average humidity. Nevertheless, it can be found rarely on rocks directly exposed to the sunlight, without the coverage of the canopy. In the present study, we follow the alterations in the subcellular organization of mesophyll cells and sugar metabolism upon desiccation of shade and sun *H. rhodopensis* plants. Composition and content of soluble carbohydrates during desiccation and rehydration were different in plants grown below the trees or on the sunny rocks. Sucrose, however, was dominating in both ecotypes. The amount of starch grains in chloroplasts was inversely related to that of sugars. Concomitantly with these changes, the number of vacuoles was multiplied in the cells. This can be explained by the development of small (secondary) vacuoles peripherally in the cytoplasm, rather than by the fragmentation of the single vacuole, proposed earlier in the literature. Accordingly, the centripetal movement of chloroplasts and other organelles may be a result of the dynamic changes in the vacuolar system. Upon rehydration, the inner vacuoles enlarged and the organelles returned to their normal position.

Keywords Vacuole formation · Soluble sugars · Resurrection plants · Desiccation tolerance

Introduction

A small group of angiosperms known as resurrection plants possess vegetative tissues that are able to tolerate severe dehydration (Gaff 1971). They could dry to equilibrium with the air humidity but resume normal function upon rehydration (Alpert 2006). Since enzymes and membranes require a hydrate coat, desiccation-tolerant cells must be able to cease metabolism and restart it upon rewatering. Extreme water stress induces mechanical stress associated with the turgor loss, oxidative stress from free radical-mediated processes, and the destabilization of macromolecular integrity (Illing et al. 2005). The loss of protoplasmic water results in considerable anatomical and ultrastructural re-organization of the leaf tissue and various metabolic and physiological changes. The molecular and biochemical investigations indicated that these organisms tolerate desiccation through a set of different mechanisms, including the downregulation of metabolism and removal of reactive oxygen species and the free radicals; the accumulation of certain proteins, such as late embryogenesis abundant or small heat shock proteins, which appear to be also

Handling Editor: Néstor Carrillo

Electronic supplementary material The online version of this article (doi:10.1007/s00709-015-0932-0) contains supplementary material, which is available to authorized users.

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Plant Mol Biol Rep (2017) 35:313–322
DOI 10.1007/s11105-017-1025-3

ORIGINAL PAPER

Drought-Responsive Gene Expression in Sun and Shade Plants of *Haberlea rhodopensis* Under Controlled Environment

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Published online: 3 February 2017

Abstract *Haberlea rhodopensis* is a homoiochlorophyllous desiccation-tolerant plant growing mostly in shaded rock rifts below the trees at very low light intensity. These shade plants are very sensitive to photoinhibition and do not survive desiccation at irradiance of 350 μmol m^{-2} s^{-1}, whereas plants growing on the top of rocks exposed to full sunlight (sun plants) can survive at even higher light intensities regularly. The aim of the present study was to establish how acclimation to different light intensities influences the expression of selected drought-responsive genes and the physiological activity during desiccation of shade and sun plants under controlled culture conditions. The photosynthetic activity was higher in sun plants not only when fully hydrated but also during dehydration. Thus, the higher photosynthetic capacity, reflected in PSII but especially in PSI activity, is accompanied by a reduced susceptibility to photodamage. For most of the genes examined, drought was the main factor in regulation; in addition, some were light modulated like genes coding for putative superoxide dismutase (SOD), ascorbate peroxidase (APX) and thioredoxin (TRX), whereby the former was almost purely light regulated. Differences between sun and shade plants concerned mainly on the time course. Whereas some genes reacted already at moderate desiccation only in sun plants (genes for monodehydroascorbate reductase (MDAR), plastidic translocase (PTL) similar to OEP16 and one of the genes, newly annotated ELIP-like, specific for *H. rhodopensis*), especially a gene for a putative UDP-glucuronic acid decarboxylase (UDP) retained its enhanced expression longer during recovery. Thus, these genes are probably especially important for survival and recovery in sun plants.

Keywords Desiccation tolerance · Gene expression · Photosynthesis · Resurrection plants

Electronic supplementary material The online version of this article (doi:10.1007/s11105-017-1025-3) contains supplementary material, which is available to authorized users.

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Introduction

Desiccation-tolerant or resurrection plants are unique among angiosperms with their ability to survive desiccation to an air-dry state. Many of these species grow in semiarid and arid countries, and a rich diversity is found in Southern Africa (Moore and Farrant 2012). The European flowering desiccation-tolerant plant species are restricted to two genera of the family Gesneriaceae—*Ramonda* and *Haberlea*. *Haberlea rhodopensis* Friv. is an endemic species that survived as a tertiary relict on the Balkan Peninsula. It is a perennial herbaceous rock plant, forming dense tufts of leaves, each rosette bearing in spring one to five flower-stalks. *H. rhodopensis* is considered a homoiochlorophyllous desiccation-tolerant plant, since it preserves its chlorophyll content during dehydration. It grows on limestone or silicate rocks, mostly in rock rifts in shaded places with high air humidity, as well as in beech and pine tree forests (Daskalova et al. 2011). *H. rhodopensis* localities show great variations in habitat characteristics. For instance, their altitude varies from 136 m to over 1400 m. There are also extreme differences in temperature, water and light conditions. In relation to the different light and water conditions, two types of *H. rhodopensis*

Г7-10

Plant Physiology and Biochemistry 125 (2018) 185–192

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Application of a diffusion model to measure ion leakage of resurrection plant leaves undergoing desiccation

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ARTICLE INFO

Keywords:
Apoplast
Electrolyte leakage
Desiccation
Haberlea rhodopensis
Ion permeability
Symplast
Vacuoles

ABSTRACT

Haberlea rhodopensis is a chlorophyll-retaining resurrection plant, which can survive desiccation to air dry state under both low light and sunny environments. Maintaining the integrity of the membrane during dehydration of resurrection plants is extremely important. In the present study, the diffusion model was improved and used for a first time to evaluate the changes in ion leakage through different cellular compartments upon desiccation of *H. rhodopensis* and to clarify the reasons for significant increase of electrolyte leakage from dry leaves. The applied diffusion approach allowed us to distinguish the performance of plants subjected to dehydration and subsequent rehydration under different light intensities. Well-hydrated (control) shade plants had lower and slower electrolyte leakage compared to control sun plants as revealed by lower values of phase amplitudes, lower rate constants and ion concentration. In well-hydrated and moderately dehydrated plants (50% relative water content, RWC) ion efflux was mainly due to leakage from apoplast. The electrolyte leakage sharply increased in severely desiccated leaves (8% RWC) from both sun and shade plants mainly due to ion efflux from symplast. After 1 day of rehydration the electrolyte leakage was close to control values, indicating fast recovery of plants. We suggest that the enhanced leakage in air-dried leaves should not be considered as damage but rather as a survival mechanism based on a reversible modification in the structure of cell wall, plasma membrane and alterations in vacuolar system of the cells. However, further studies should be conducted to investigate the changes in cell wall/plasma membrane to support this conclusion.

1. Introduction

Resurrection or desiccation-tolerant (DT) plants are a small group of vascular plants that can tolerate desiccation of their vegetative tissues up to air-dry state and after re-watering they recover their normal physiological functions (Alpert, 2005). Two types of resurrection plants can be distinguished: homoiochlorophyllous (HDT) and poikilochlorophylous (PDT). The HDTs retain their chlorophyll and photosynthetic apparatus on desiccation, whereas in PDTs desiccation results in the loss of chlorophyll, which must be re-synthetized following remoistening. The PDT strategy is based on the dismantling of internal chloroplast structure by an ordered deconstruction process during drying, and its re-synthesis upon rehydration (Tuba et al., 1998; Farrant, 2000).

Plant photosynthetic apparatus is very sensitive and liable to injury. It is well known that drought stress inhibits photosynthesis process due to stomatal closure and imbalance between light capture and energy utilization (Chaves et al., 2009). Since HDT plants keep their chlorophyll upon dehydration, these molecules continue to harvest energy, but cannot use it for photosynthesis and this can lead to oxidative burst in chloroplasts due to transfer of electrons from electron transport chains to oxygen thus leading to production of reactive oxygen species (ROS) (Lawlor and Cornic, 2002; Tuba, 2008). Other source of ROS production are the respiratory electron transport chain of mitochondria

Abbreviations: A_1, the relative part of electrolytes in apoplast; A_2, the relative part of electrolytes in symplast; A_3, the relative part of electrolytes in vacuoles; *C(T)*, ion concentration at the moment *T*; *dC(0)/dt*, initial leakage rate; *dC(T)/dt*, rate of leakage at the moment *T*; HDT, homoiochlorophyllous desiccation tolerant plants; κ/κ_{max}, relative change in electrolyte concentration in the outer solution; RWC, relative water content; ROS, reactive oxygen species; τ_1, characteristic time constants of ion movement through apoplast; τ_2, characteristic time constants of ion movement through symplast; τ_3, characteristic time constants of ion movement through tonoplast

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https://doi.org/10.1016/j.plaphy.2018.02.008
Received 6 December 2017; Received in revised form 4 February 2018; Accepted 6 February 2018
Available online 13 February 2018
0981-9428/

Environmental and Experimental Botany 178 (2020) 104157

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Freezing tolerance of photosynthetic apparatus in the homoiochlorophyllous resurrection plant *Haberlea rhodopensis*

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ARTICLE INFO

Keywords:
Blue Native-PAGE
Epidermal channels
Frost
Desiccation
Pigment-protein complexes
Photosynthetic proteins

ABSTRACT

Haberlea rhodopensis is unique among homoiochlorophyllous resurrection plants with its ability to tolerate also low temperatures at temperate climate. This study was carried out to elucidate the response and acclimation ability of photosynthetic apparatus to cold and the capacity to tolerate freezing temperatures. Cold acclimated plants experienced short-term freezing conditions under controlled-environment in climatic chamber and also long-term subzero winter temperatures in an *ex-situ* natural environment. Our results indicated strong freezing tolerance after cold acclimation. In fact, fresh leaves were exposed to and survived freezing stress under both controlled and *ex-situ* conditions, thus confirming their appropriate cold acclimation. Freezing temperatures induced desiccation of leaves and the corresponding ultrastructural changes in mesophyll cells under *ex situ* environmental conditions. However, while the complete rearrangement in the cells commenced at 20 % RWC under drought stress, this process started already at 60 % RWC under freezing conditions. The presence of epidermal channels on both leaf sides was observed and their role for the fast water loss is proposed. It was found that freezing-induced desiccation, reversible downregulation of photosynthesis, readjustments in the abundance/organization of the pigment protein complexes and the main photosynthetic proteins together with increased thermal energy dissipation during chilling and freezing temperatures enable plants to survive harsh winter conditions and their fast recovery with the onset of spring. Low temperature responses are discussed in comparison to desiccation-induced changes to point out both common and specific features.

1. Introduction

Low temperature is one of the most important environmental stress factors limiting plant growth, productivity and distribution both on the long term exposure and on temporary events of extreme thermal phenomenon, i.e. cold waves (FAO, 2011), which are increasing in intensity under the current climate change (Intergovernmental Panel on Climate Change (IPCC, 2014). Cold stress (temperature above 0 °C), occurs in the absence of ice nucleation in the plant cell and induces alterations in almost all growth and metabolic processes, including photosynthesis, redox reactions, carbohydrate metabolism, transcription and protein expression (Ruelland et al., 2009; Gołębiowska-Pikania et al., 2017). Species of tropical origin may be seriously injured when exposed to temperatures below 5–10 °C, whereas the exposure of taxa from temperate climate to low, non-freezing temperatures generally results in a substantial increase in their freezing tolerance (Guy, 1990; Thomashow, 1999; Kaplan et al., 2007). Freezing stress, caused by subzero temperatures, results in the formation of ice crystals in the apoplast, and in turn it can lead to cellular dehydration, lysis damage of plasma membranes and necrosis (Thomashow, 1998). In addition to the mechanical stress, plants exposed to non-optimal low temperatures are also endangered by oxidative stress due to an imbalance between production and scavenging of reactive oxygen species (ROS).

In most regions of the temperate zone, freezing tolerance is crucial for the survival of perennial species, as well as of annual and overwintering herbaceous plants. The ability of plants to survive freezing temperature largely depends on their acclimation capacity to cold temperatures (Chen and Thelen, 2016). An increased low thermal limits are generally present in taxa from temperate climate regions with freezing tolerance triggered by exposure to low, non-injuring

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https://doi.org/10.1016/j.envexpbot.2020.104157
Received 14 November 2019; Received in revised form 28 May 2020; Accepted 11 June 2020
Available online 20 June 2020

Г7-12

Physiol Mol Biol Plants (May 2021) 27(5):1119–1133
https://doi.org/10.1007/s12298-021-00998-0

RESEARCH ARTICLE

The role of antioxidant defense in freezing tolerance of resurrection plant *Haberlea rhodopensis*

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Received: 19 November 2020 / Revised: 19 February 2021 / Accepted: 20 April 2021 / Published online: 7 May 2021

Abstract *Haberlea rhodopensis* Friv. is unique with its ability to survive two extreme environmental stresses—desiccation to air-dry state and subzero temperatures. In contrast to desiccation tolerance, the mechanisms of freezing tolerance of resurrection plants are scarcely investigated. In the present study, the role of antioxidant defense in the acquisition of cold acclimation and freezing tolerance in this resurrection plant was investigated comparing the results of two sets of experiments—short term freezing stress after cold acclimation in controlled conditions and long term freezing stress as a part of seasonal temperature fluctuations in an outdoor ex situ experiment. Significant enhancement in flavonoids and anthocyanin content was observed only as a result of freezing-induced desiccation. The total amount of polyphenols increased upon cold acclimation and it was similar to the control in post freezing stress and freezing-induced desiccation. The main role of phenylethanoid glucoside, myconoside and hispidulin 8-C-(2-O-syringoyl-b-glucopyranoside) in cold acclimation and freezing tolerance was elucidated. The treatments under controlled conditions in a growth chamber showed enhancement in antioxidant enzymes activity upon cold acclimation but it declined after subsequent exposure to −10 °C. Although it varied under ex situ conditions, the activity of antioxidant enzymes was high, indicating their important role in overcoming oxidative stress under all treatments. In addition, the activity of specific isoenzymes was upregulated as compared to the control plants, which could be more useful for stress counteraction compared to changes in the total enzyme activity, due to the action of these isoforms in the specific cellular compartments.

Keywords Anthocyanins · Antioxidant enzymes · Cold acclimation · Freezing-induced desiccation · Polyphenols · UV-absorbing flavonoids

Introduction

Haberlea rhodopensis (family: *Gesneriaceae*) belongs to the group of desiccation tolerant plant also termed as resurrection plants that can survive desiccation to air-dry climates. This endemic species, which has survived as a tertiary relict on the Balkan Peninsula, is characterized by high ecological plasticity (Daskalova et al. 2011). In contrast to most other resurrection plants, *H. rhodopensis* can withstand freezing temperatures during winter. However, exposure of plants to sub-zero temperatures resulted reduced relative water content (RWC) in leaves and thereafter they desiccate very quickly to an air-dry condition (Mihailova et al. 2020). Thus, similar to drought, freezing stress also causes dehydration of plants so they can survive the harsh winter conditions in dry conditions.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12298-021-00998-0.

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Г7-13

International Journal of
Molecular Sciences

MDPI

Article

Protective Strategies of *Haberlea rhodopensis* for Acquisition of Freezing Tolerance: Interaction between Dehydration and Low Temperature

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Abstract: Resurrection plants are able to deal with complete dehydration of their leaves and then recover normal metabolic activity after rehydration. Only a few resurrection species are exposed to freezing temperatures in their natural environments, making them interesting models to study the key metabolic adjustments of freezing tolerances. Here, we investigate the effect of cold and freezing temperatures on physiological and biochemical changes in the leaves of *Haberlea rhodopensis* under natural and controlled environmental conditions. Our data shows that leaf water content affects its thermodynamical properties during vitrification under low temperatures. The changes in membrane lipid composition, accumulation of sugars, and synthesis of stress-induced proteins were significantly activated during the adaptation of *H. rhodopensis* to both cold and freezing temperatures. In particular, the freezing tolerance of *H. rhodopensis* relies on a sucrose/hexoses ratio in favor of hexoses during cold acclimation, while there is a shift in favor of sucrose upon exposure to freezing temperatures, especially evident when leaf desiccation is relevant. This pattern was paralleled by an elevated ratio of unsaturated/saturated fatty acids and significant quantitative and compositional changes in stress-induced proteins, namely dehydrins and early light-induced proteins (ELIPs). Taken together, our data indicate that common responses of *H. rhodopensis* plants to low temperature and desiccation involve the accumulation of sugars and upregulation of dehydrins/ELIP protein expression. Further studies on the molecular mechanisms underlying freezing tolerance (genes and genetic regulatory mechanisms) may help breeders to improve the resistance of crop plants.

Keywords: resurrection plants; freezing tolerance; desiccation; carbohydrates; fatty acids; protective proteins

Citation: Georgieva, K.; Mihailova, G.; Fernández-Marín, B.; Bertazza, G.; Govoni, A.; Arzac, M.I.; Laza, J.M.; Vilas, J.L.; García-Plazaola, J.I.; Rapparini, F. Protective Strategies of *Haberlea rhodopensis* for Acquisition of Freezing Tolerance: Interaction between Dehydration and Low Temperature. *Int. J. Mol. Sci.* **2022**, *23*, 15050. https://doi.org/10.3390/ijms232315050

Academic Editor: Tibor Janda

Received: 29 September 2022
Accepted: 28 November 2022
Published: 30 November 2022

1. Introduction

Low temperatures are one of the most harmful abiotic factors affecting the growth and survival of overwintering plants. These species acclimate to seasonal variations in temperature by adjusting their metabolism during autumn. It is well known that exposure of temperate plants to non-freezing low temperatures increases their freezing tolerance [1]. During cold acclimation, the metabolism is redirected towards the synthesis of cryoprotective molecules, including soluble sugars, sugar alcohols, and other low-molecular-weight

Γ7-14

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Protein Changes in Shade and Sun *Haberlea rhodopensis* Leaves during Dehydration at Optimal and Low Temperatures

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Abstract: *Haberlea rhodopensis* is a unique resurrection plant of high phenotypic plasticity, colonizing both shady habitats and sun-exposed rock clefts. *H. rhodopensis* also survives freezing winter temperatures in temperate climates. Although survival in conditions of desiccation and survival in conditions of frost share high morphological and physiological similarities, proteomic changes lying behind these mechanisms are hardly studied. Thus, we aimed to reveal ecotype-level and temperature-dependent variations in the protective mechanisms by applying both targeted and untargeted proteomic approaches. Drought-induced desiccation enhanced superoxide dismutase (SOD) activity, but FeSOD and Cu/ZnSOD-III were significantly better triggered in sun plants. Desiccation resulted in the accumulation of enzymes involved in carbohydrate/phenylpropanoid metabolism (enolase, triosephosphate isomerase, UDP-D-apiose/UDP-D-xylose synthase 2, 81E8-like cytochrome P450 monooxygenase) and protective proteins such as vicinal oxygen chelate metalloenzyme superfamily and early light-induced proteins, dehydrins, and small heat shock proteins, the latter two typically being found in the latest phases of dehydration and being more pronounced in sun plants. Although low temperature and drought stress-induced desiccation trigger similar responses, the natural variation of these responses in shade and sun plants calls for attention to the pre-conditioning/priming effects that have high importance both in the desiccation responses and successful stress recovery.

Keywords: drought stress; frost-induced desiccation; LC-MS/MS; proteomics; resurrection plants

Citation: Mihailova, G.; Solti, Á.; Sárvári, É.; Hunyadi-Gulyás, É.; Georgieva, K. Protein Changes in Shade and Sun *Haberlea rhodopensis* Leaves during Dehydration at Optimal and Low Temperatures. *Plants* **2023**, *12*, 401. https://doi.org/10.3390/plants12020401

Academic Editors: Szabolcs Rudnóy, Sruthy Maria Augustine and Kaushal Kumar Bhati

Received: 28 November 2022
Revised: 10 January 2023
Accepted: 12 January 2023
Published: 15 January 2023

1. Introduction

Resurrection plants represent a small group of angiosperms that possess the ability to survive desiccation to an air-dry state and recover to normal metabolic functions upon rehydration [1]. *Haberlea rhodopensis*, like other European species such as *Ramonda serbica*, *Ramonda nathaliae*, *Ramonda myconi*, and *Jankaea heldreichii*, is a desiccation-tolerant member of the Gesneriaceae family [2–5]. The homoiochlorophyllous resurrection plant *H. rhodopensis* is a Tertiary relict on the Balkan Peninsula [6,7]. In its natural habitat in the Rhodope Mountains, *H. rhodopensis* colonizes rock surfaces at an altitude from 136 to near 1600 m a.s.l., and the sites of occurrence extremely differ in temperature, humidity, and light conditions [8]. Although the taxon has a clear preference for shady habitats (e.g., north-faced limestone or undercanopy silicate rocks and maximum photosynthetically active photon flux density (PPFD) of 25–30 µmol m^{-2} s^{-1}; these are referred to as shade plants), *H. rhodopensis* also colonizes rock clefts directly exposed to sunlight (maximum PPFD of 1500–1700 µmol m^{-2} s^{-1}; referred to as sun plants). In the natural habitat, both shade and sun plants undergo desiccation in response to drought stress due to the lack of

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Acquisition of Freezing Tolerance of Resurrection Species from Gesneriaceae, a Comparative Study

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Abstract: Resurrection plants have the unique ability to restore normal physiological activity after desiccation to an air-dry state. In addition to their desiccation tolerance, some of them, such as *Haberlea rhodopensis* and *Ramonda myconi*, are also freezing-tolerant species, as they survive subzero temperatures during winter. Here, we compared the response of the photosynthetic apparatus of two other Gesneriaceae species, *Ramonda serbica* and *Ramonda nathaliae*, together with *H. rhodopensis*, to cold and freezing temperatures. The role of some protective proteins in freezing tolerance was also investigated. The water content of leaves was not affected during cold acclimation but exposure of plants to −10 °C induced dehydration of plants. Freezing stress strongly reduced the quantum yield of PSII photochemistry (Y(II)) and stomatal conductance (g_s) on the abaxial leaf side. In addition, the decreased ratio of F_v/F_m suggested photoinhibition or sustained quenching. Freezing-induced desiccation resulted in the inhibition of PSII activity, which was accompanied by increased thermal energy dissipation. In addition, an increase of dehydrins and ELIPs was detected, but the protein pattern differed between species. During recovery, the protein abundance decreased and plants completely recovered their photosynthetic activity. Thus, our results showed that *R. serbica*, *R. nathaliae*, and *H. rhodopensis* survive freezing stress due to some resurrection-linked traits and confirmed their freezing tolerance.

Keywords: freezing-induced desiccation; chlorophyll fluorescence; stomatal conductance; pigments; dehydrins; ELIP; *Ramonda serbica*; *Ramonda nathaliae*; *Haberlea rhodopensis*

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Citation: Mihailova, G.; Gashi, B.; Krastev, N.; Georgieva, K. Acquisition of Freezing Tolerance of Resurrection Species from Gesneriaceae, a Comparative Study. *Plants* **2023**, *12*, 1893. https://doi.org/10.3390/plants12091893

Academic Editors: Massimiliano Tattini, Antonella Gori and Luana Beatriz dos Santos Nascimento

Received: 29 March 2023
Revised: 28 April 2023
Accepted: 4 May 2023
Published: 5 May 2023

1. Introduction

The rare phenomenon of desiccation tolerance in the vegetative tissues of vascular plants is possessed by so-called resurrection plants. They have the unique ability to restore normal physiological activity after desiccation to an air-dry state [1]. Drought stress inhibits photosynthesis mainly due to stomata closure, thus leading to mesophyll limitations [2] and disruption of the balance of energy capture and utilization via carbon metabolism [3]. As a result, reactive oxygen species (ROS) are generated and oxidative stress occurs in plant cells, leading to serious damage to DNA, proteins, and lipids [4]. Moreover, dehydration causes cell wall shrinkage and following plasma membrane rupture. Thus, resurrection plants have to cope with mechanical, structural, oxidative and metabolic stresses [5]. They evolve different strategies to preserve the integrity of membranes, macromolecules, and photosynthetic machinery by minimizing ROS production in the cells. Downregulation of photosynthesis, enhanced thermal energy dissipation, rearrangement of cell structure, upregulation of antioxidant defense system, synthesis of compatible solutes, and different protective proteins are part of those strategies of plants to cope with drought stress [4–6].

In addition to their desiccation tolerance, two of the European resurrection gesneriads, *Haberlea rhodopensis* and *Ramonda myconi*, are also freezing-tolerant species, as they survive

Г8-01

37 Drought Tolerance of Photosynthesis

Katya Georgieva and Gergana Mihailova

CONTENTS

37.1 INTRODUCTION

Drought is one of the major environmental factors that inhibits many metabolic processes and constrains plant growth and crop productivity. The ongoing global warming and current climate changes are enlarging the land areas where plants experience water deficit. Understanding the responses of plants to their external environment is of importance with respect to basic research, but it is also an attractive target for improving stress tolerance [1]. Thus, our understanding of the drought adaptation mechanisms is of importance to meet the goal of increased plant productivity under the projected critical global scenarios that are related to water availability.

The most severe form of water deficit is desiccation, when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. Plants are very sensitive to desiccation during the vegetative phase of their life cycle, and very few plants acquire desiccation tolerance in the vegetative tissues. These include a small group of angiosperms, termed *resurrection plants* [2], which are capable of surviving water loss to an air-dry state. Resurrection plants are mostly poikilohydrous, which means that their water content adjusts with the relative humidity in the environment. They are able to stay in the dehydrated state until water becomes available and allows them to rehydrate and to resume full physiological activities [3–5]. In this small group of plants, the mature leaves, roots, and shoots can lose up to 95% of their water. Vegetative desiccation tolerance in angiosperms is comparatively rare, with approximately 300–400 species being reported as desiccation tolerant [6,7]. It has been suggested that desiccation tolerance is connected with size limitation, since all examples of desiccation tolerant flowering plants do not exceed a certain height [8]; perhaps the largest known resurrection plant is the small woody shrub *Myrothamnus flabellifolia* [9]. Most resurrection plants are herbaceous plants. Resurrection plants are found in ecological niches with limited seasonal water availability, preferentially on rocky outcrops in semiarid and arid countries, and a rich diversity is found in Southern Africa [6,7,10]. Resurrection species are found also in the Balkans (*Haberlea rhodopensis, Ramonda* spp.), China (*Boea hygrometrica*), Australia (*Sporobolus* and *Eragrostis* spp.), North and Central America (*Tortula ruralis*), and South America (*Pleurostima purpurea*) [11,12]. Resurrection plants have been identified within the angiosperms both among monocotyledonous and dicotyledonous plants, but no desiccation tolerant gymnosperms or trees have been reported yet [5,13,14].

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 [12,15,16]. Bryophytes are considered as *fully desiccation-tolerant* plants [17] and can withstand very fast drying. The tolerance to desiccation of these plants is based on cellular protection upon dehydration coupled with the repair of cell damage during rehydration [18,19]. The vascular species, designated as *modified desiccation-tolerant* plants, can survive desiccation only if the drying rate is slow. In these plants, the tolerance relies mainly on cellular protection during dehydration [12,20].