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

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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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long-term range (Chow et al., 1990; Kanervo et al., 2005; Walters, 2005; Eberhard et al., 2008). Variations in the interactions of thylakoid complexes give remarkable contribution to the protective mechanisms acting under various stresses (Damkjær et al., 2009).

Resurrection plants have a unique ability to survive dehydration to the air-dry state. *Haberlea rhodopensis* Friv. (Gesneriaceae) is a rare Balkan endemic and Tertiary relict plant that belongs to the homoiochlorophyllous (Chl-retaining) resurrection type as it can preserve most of its photosynthetic apparatus during desiccation. *H. rhodopensis* plants growing in deep shadow under natural conditions were shown to be very sensitive to photoinhibition (Georgieva and Maslenkova, 2006). In fact, desiccation at an irradiance of 350 $\mu mol\ m^{-2}\ s^{-1}$ induced irreversible changes in the photosynthetic apparatus, and mature leaves did not recover after rehydration (Georgieva et al., 2008). On the other hand, recent studies revealed unexpected ecological plasticity of *H. rhodopensis* in natural habitats (Daskalova et al., 2011). Besides the shady habitats, several habitats of high irradiance were discovered recently, where plants grow on rocks directly exposed to the sunlight, out of the forest coverage and with low air humidity. Our previous results showed that the membrane integrity of sun plants was well protected, and regardless of the higher malondialdehyde content measured in the well-hydrated sun *H. rhodopensis* plants compared to shade ones, desiccation of plants at high light did not cause additional oxidative damage (Georgieva K et al., 2012). The higher photosynthetic activity of well-hydrated sun plants reduced the susceptibility of photodamage. In addition, a significantly lower proportion of light was allocated to photochemistry during desiccation at high irradiance due to the different protective mechanisms (Solti et al., 2014).

While Chl–protein complexes play important role in acclimation of photosynthesis, data on the dehydration and rehydration-induced changes in the thylakoid complexes of resurrection plants, including *H. rhodopensis*, are sporadic, and mostly studied on transcriptome rather than on proteome level (Dinakar et al., 2012; Georgieva T et al., 2012; Gechev et al., 2013). Concerning the thylakoid complexes, no or only slight changes in the Chl–protein pattern were found in *Boea hygrometrica* (Gesneriaceae) and *H. rhodopensis* using native PAGE (Deng et al., 2003; Georgieva et al., 2007, 2010). Some increase in the antenna size of a sucrose-gradient fraction containing PSI was observed in *H. rhodopensis* after desiccation (Georgieva et al., 2009). Preliminary experiments were conducted on *H. rhodopensis* chloroplasts by Blue-Native polyacrylamide gel electrophoresis (BN–PAGE) (Mladenov et al., 2013). In addition, several stress-induced proteins were discovered in *Craterostigma plantagineum* (Alamillo and Bartels, 2001). Reduced abundance of *Lhc* transcripts in *Sporobolus stapfianus* (Blomstedt et al., 1998) and in *C. plantagineum* (Suarez Rodriguez et al., 2010), a lower amount of *Lhcb1* in *H. rhodopensis* (Gechev et al., 2013), and slight and significant decrease in the amount of PsaA/B and D2 proteins, respectively (Mihailova et al., 2011) were also reported. However, detailed information on the remodelling of thylakoids during desiccation and rehydration is not available.

The goal of this study is to reveal how a species that generally adapted to low-light conditions can acclimate to high light at population level. What kind of morphological, structural and thylakoid-level alterations play a role in this acclimation, and how they affect or contribute to the desiccation tolerance mechanisms of *H. rhodopensis* at different habitats? To answer these questions, we analyzed the ultrastructure, Chl–protein complex organization and steady state low-temperature fluorescence properties of thylakoids in sun and shade populations under desiccation and rehydration as signs of the structural status of the photosynthetic apparatus, the most sensitive component of the Chl-retaining resurrection plants.

Materials and methods

Plant material

Experiments were conducted on *Haberlea rhodopensis* Friv. plants growing in Rhodope Mountains, South-West Bulgaria (N41°52.231; E024°36.171) at 1000–1200 m a.s.l. To study the effect of long-term growth and desiccation at high light intensity we selected plants growing on northward facing slopes of sun exposed limestone rocks ('sun' plants). They receive full sunlight, 1500–1700 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation, at midday in June that results in a leaf-level temperature of 30–37 °C and relative air humidity of about 15–30%. In order to investigate the response to long-term growth and desiccation at low irradiances we selected understory plants growing in deeply shaded rock-crevice habitats ('shade' plants). They are exposed to a light intensity of approximately 25 $\mu mol\ m^{-2}\ s^{-1}$ at midday in June that results in leaf-level temperature of 21–25 °C and a relative humidity of 40–45%. Light intensity was measured at the surface of the collected plants by QSPAR Quantum Sensor (Hansatech, United Kingdom). Leaf temperature and relative humidity values were detected by a Pocket Profi-Thermohygrometer (TFA, Germany).

Adult rosettes of similar size and appearance of well-hydrated and desiccated plants with approximately 90, 50 and 8% relative water content (RWC) were collected from their natural habitats without damaging either the leaves or the roots, and were transferred to the laboratory. Experiments were conducted on fully expanded mature leaves of well-hydrated (90–95% RWC – 90), moderately (45–55% RWC – 50) and severely dehydrated plants (6–8% RWC – 8) as well as on leaves of dry plants after one (50–60% RWC – R1) or six days (90–95% RWC – R6) of rehydration. Plants were rehydrated under laboratory conditions by watering them in a modified desiccator. The water at the bottom of the desiccator was pumped up, thus ensuring a permanent high humidity level.

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 the percentage of water content in dehydrated tissue compared to water-saturated tissues, using the equation:

$$RWC\,(\%) = \frac{(\text{fresh weight} - \text{dry weight})\,100}{(\text{saturated weight} - \text{dry weight})}.$$

Saturated weight was measured on leaf discs maintained 16 h at 4 °C in the dark floating on water.

Thylakoids were isolated from mature leaves, the size and appearance of which was characteristic to either sun or shade populations. Sub-samples from the leaves were separated and frozen in liquid nitrogen for pigment analyses or embedded for electron microscopy.

Thylakoid proteomics

Thylakoid membranes were prepared according to Georgieva et al. (2009). The Chl content of leaves (expressed on a dry weight basis) and thylakoid fractions was determined spectrophotometrically in 80% acetone by a UV–vis spectrophotometer (UV-1601, Shimadzu, Japan) using the equations of Porra et al. (1989).

To separate thylakoid complexes, first dimension electrophoresis was performed under native conditions by BN–PAGE (Kügler et al., 1997) using 5–12% w/v acrylamide gradient gels (Mini-Protean, BioRad). The thylakoids were washed in 50 mM Bis–Tris–HCl (pH 7.0) containing 330 mM sorbitol and 250 $\mu g\ mL^{-1}$ Pefabloc, and solubilized (0.5 mg Chl mL^{-1}) with 50 mM Bis–Tris–HCl (pH 7.0), 750 mM aminocaproic acid, 0.5 mM EDTA, 250 $\mu g\ mL^{-1}$ Pefabloc and 2% (w/v) *n*-dodecyl-β-D-maltoside on ice for 30 min. After 15 min centrifugation with 18,000 × *g* at