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

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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 $\mu mol\ m^{-2}\ s^{-1}$) [7]. However, desiccation at irradiance of 350 $\mu mol\ m^{-2}\ s^{-1}$ 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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against drought stress [9]. Under mild dehydration (RWC > 50%) in low light, net CO_2 assimilation decline was largely influenced by stomatal closure in shade population of *H. rhodopensis* [10]. Under strong desiccation (RWC < 50%), decline in photochemical activity of photosystem II (PSII) also decreases the activity of photosynthesis by affecting linear electron flow [7,10]. Dehydration to an air-dried state led to a slight decrease in the chlorophyll (Chl) content of leaves [10,11]. Concerning Chl-proteins, moderate lowering in the amount of D1, D2, PsbS and PsaA/B proteins and a relative increase of Lhc polypeptides were observed [12]. β-carotene content was only slightly enhanced compared to the fully hydrated leaves, while the zeaxanthin level increased strongly (F. Rapparini, personal communication).

Light exposure together with the inhibition of photosynthetic electron transport during dehydration is one of the main challenges in desiccation tolerance. Increased synthesis of phenolics during desiccation of *H. rhodopensis* leaves may contribute to drought resistance and recovery [10]. Accumulation of dense compounds (possibly phenolics) in the thylakoid lumen was proposed as a protective mechanism during desiccation and recovery in low-light-grown plants [13]. Reversible modifications in PSII, and enhanced probability for thermal ways of energy dissipation during desiccation also contributed to drought resistance of *H. rhodopensis* and its fast recovery after rehydration [10]. However, adaptive mechanisms of *H. rhodopensis* plants living under high light conditions have not been studied yet in detail.

Energy dissipation routes in chloroplasts are usually studied by measuring light induced variations in the >685 nm Chl *a* fluorescence with PAM method. Red region of this fluorescence (685–690 nm) is originated from PSII, while far-red part (near 730–740 nm) comes from PSI, which latter have a negligible [less than 10%] contribution at room temperature [14,15]. However, there are many factors that influence the red to far-red ratio. The short wavelength PSII fluorescence at 690 nm overlaps with the absorption maximum of Chl *a* (at 680 nm), so the ratio between the red and far-red maxima depends on the Chl *a* concentration of the leaf tissue: reabsorption of the emitted light results in a decrease of the ratio F690/F735 with increasing Chl *a* content [16]. Furthermore, the percentage of the contribution of PSI fluorescence above 700 nm depends on the activity of photosynthetic electron transport. Pfündel [14] estimated that it reaches a proportion of about 30–40% of the minimal Chl *a* fluorescence yield (F_o) in C_3 plants, and 40–60% in C_4 plants. In addition, PSI seems to be much more resistant to strong light stress than PSII [17]. The F690/F735 ratio in steady state fluorescence spectra could be used to assess Chl reabsorption changes in the photosynthetic apparatus [18], developmental processes of leaves [19], and stress events [20,21]. Therefore, detection of the Chl *a* fluorescence by a two-wavelength method (red and far-red detection) is a promising new way to reveal changes in the stage of the photosynthetic apparatus.

The aim of the present study is to show the role of non-photochemical quenching routes in the excitation energy allocation under desiccation of *H. rhodopensis* leaves collected from natural habitats of low and high irradiance by using the two-wavelength detection with a new Intelligent FluoroSensor (IFS) apparatus. Basic functions of the (net enabled and wireless) IFS apparatus has already been shown [22], but here, its more complete functionality was utilised on a more complex species of interest.

2. Materials and methods

2.1. Plant material

Well-hydrated *Haberlea rhodopensis* Friv. plants were collected in Rhodope Mountains (Bulgaria, at altitude ca. 1000 m) at two neighbouring natural habitats: shade plants grown under very low average daily irradiance (about 25 μmol m^{-2} s^{-1} in summer) were collected from rocks below trees whereas sun plants grown under high average daily irradiance (about 1500 μmol m^{-2} s^{-1} in summer) were collected from northward facing slopes of sun exposed limestone rocks. Leaves of adult rosettes from the same locality and of similar size and appearance were selected for the experiments. Leaves detached from fully rehydrated plants (kept under wet conditions in darkness until the start of the experiment) were dehydrated at ambient (~50%) air humidity in darkness under a plastic cover to slow down the desiccation process.

2.2. Chlorophyll fluorescence induction measurements by the IFS system

Chl fluorescence induction measurements were performed by wireless IFS system device constructed by Barócsi [22]. The sensor is using four different light sources: (i) A far-red light source with an irradiance of 8.5 W m^{-2} at 720 nm and duration of 5 s to excite PSI and opening the PSII reaction centres before measuring F'_o fluorescence [23]. (ii) A red diode laser illuminating at 635 nm used at 37.5% of its maximal power as an actinic source. This yields a high level actinic PPFD of 820 μmol m^{-2} s^{-1}. (iii) The red laser diode with its maximal PPFD of 2200 μmol m^{-2} s^{-1} in combination with the blue LED light source emitting at 405 nm with PPFD of 270 μmol m^{-2} s^{-1} is used to produce high intensity, excitation flashes ('saturating light') with pulse duration of 1 s. (iv) A 635 nm LED modulated at 25 kHz with pulse duration of 1 μs having a PPFD of 7.7 μmol m^{-2} s^{-1} was used as a pulsed light source for the PAM technique. The PPFD values given are measured on a 4.5 mm diameter detector placed coaxially at the sample surface of the instrument.

The instrument can be used with three different detectors. The first detector is for a broadband channel from 680 nm to 740 nm, measuring the variable fluorescence with the pulse amplitude modulated (PAM) technique. The two other channels are measuring the total (unmodulated, or DC) fluorescence at two different narrow wavebands of 10 nm, at 690 nm and at 735 nm, respectively. The sensor is also equipped with a dual channel (ambient + leaf surface) infrared temperature sensor.

At a given stage of leaves, the Chl fluorescence induction measurement protocol started with a 120 s dark condition. F_o level of fluorescence was determined after a short far-red illumination with the light source (i). The maximum fluorescence yields (F_p): F_m in the dark-adapted state, F'_m in the light-adapted state, and F''_m (with response $F''_m < F_m$) applied in 1 min after removing the actinic light (that is at 230 s) were determined by excitation flashes of light source (iii) which saturated the PSII electron transport by closing all PSII traps. F''_m may be used to characterise the fast component of the non-photochemical quenching. Maximal efficiency of PSII centres was determined as $F_v/F_m = (F_m - F_o)/F_m$ [24,25]. Light adaptation started by switching on light source (ii) and fluorescence (F_t) was recorded during the whole time course. Light adapted quasi steady-state fluorescence (F_s) and maximum fluorescence yield in the light adapted state (F'_m) were determined after 180 s light adaptation according to the IFS protocol, designed for quick measurements. Previous studies showed that 180 s measurements are sufficient for the exact determination of steady-state parameters [22]. For excitation energy allocation experiments, quenching parameters were calculated according to Hendrickson et al. [26] modified by Sárvári et al. [27]:

$$\Phi_{PSII} = \left(1 - \frac{F_s}{F'_m}\right) * \left(\frac{F_v/F_m}{F_{vM}/F_{mM}}\right) \quad (1)$$

$$\Phi_{NPQ} = \left(\frac{F_s}{F'_m} - \frac{F_s}{F_m}\right) * \left(\frac{F_v/F_m}{F_{vM}/F_{mM}}\right) \quad (2)$$