

Effect of Desiccation of the Resurrection Plant *Haberlea Rhodopensis* at High Temperature on the Photochemical Activity of PSI and PSII

Gergana Mihailova*, Snejana Petkova, Detelin Stefanov, Katya Georgieva

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria.

*Corresponding author. Tel. No. +359 2 979 2688; Fax No. +359 2 8739952; E-mail: gkm_rw@abv.bg.

Abstract: Changes in the photochemical activity of the homoiochlorophyllous poikilohydric plant *Haberlea rhodopensis* under dehydration at high temperature was investigated. Plants, growing under low irradiance in their natural habitat, were desiccated to air-dry state at a similar light intensity (about $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) under optimal (23/20 °C) or high (38/30 °C) day/night temperature. Water deficit reduced photochemical activity of PSII and PSI. The results showed that desiccation of *Haberlea rhodopensis* at high temperature had more limiting effects than desiccation at optimal temperature. However, the damage was limited to a level where repair was still possible and thus plants fully recovered after 7 days of rehydration.

Keywords: Chlorophyll fluorescence; Drought stress; *Haberlea rhodopensis*; High temperature; Photosynthesis

Introduction

Most higher plants are unable to survive desiccation to an air-dried state but a small group of angiosperms known as poikilohydric or resurrection plants can tolerate extreme dehydration and some are even able to equilibrate the leaves with air to 0% (v/v) relative humidity and then regain normal function after rehydration (Gaff, 1971). Resurrection plants may be subdivided into two groups: homoiochlorophyllous desiccation tolerant plants (HDT), which retain their chlorophyll during drying and poikilochlorophyllous desiccation tolerant plants (PDT), which lose chlorophyll on drying (Tuba, 1998). Drought stress is known to inhibit photosynthetic activity of the plants due to an imbalance between light capture and its utilization (Foyer and Noctor, 2000). Under natural conditions drought is often accompanied by high temperature, which has a strong impact on the vitality of plants. Important high temperature effects on photosynthesis are the inactivation of thylakoid membrane reactions and damage to the enzymes involved in photosynthetic carbon metabolism (Berry and Björkman, 1980). *Haberlea rhodopensis* Friv. (Gesneriaceae) is a rare resurrection plant of the northern hemisphere, originating from the Balkan

Peninsula as an endemic and relict species of the Tertiary period. It is considered as a homoiochlorophyllous resurrection plant, since it preserves its chlorophyll content during dehydration. *Haberlea* grows in regions with severe water scarcity and simultaneous high temperature. The aim of the present study was to investigate the effect of high temperature during desiccation on the photochemical activity of PSI and PSII.

Materials and Methods

Well-hydrated *Haberlea rhodopensis* plants were collected from their natural habitat where they grow on rocks below trees under very low irradiance. Plants were subjected to drought stress by withholding irrigation either at 23/20 °C or 38/30 °C day/night temperature, irradiance of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 12 h photoperiod, and relative humidity of 60%. After desiccation to air-dry state, the plants were rehydrated. Control plants, kept at 23/20 °C or 38/30 °C were regularly watered during the experiment. The relative water content (RWC) of *Haberlea* 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:

$$\text{RWC (\%)} = (m_{\text{fresh}} - m_{\text{dry}}) \times 100 / (m_{\text{saturated}} - m_{\text{dry}})$$

Chlorophyll fluorescence emission from the upper leaf surface was measured with a pulse amplitude modulation fluorometer (PAM 101–103, Walz, Effeltrich, Germany). Induction kinetics were registered and analyzed with a program FIP 4.3, written by Tyystjarvi and Karunen (1990). The redox state of P700 was monitored *in vivo* as 810/860 nm absorption changes. A Walz ED 700DW-E emitter/detector unit was connected to a PAM 101E main control unit (Klughammer and Schreiber, 1998). Leaf absorbance changes at 810 nm were measured as a function of increasing fluence rates of far-red light (FR) to calculate a 'saturation constant', K_s , representing the far-red irradiance at which half of the maximum absorbance change ($\Delta A_{810\text{max}}/2$) was reached. Far-red intensities were varied in 11 steps. The value of K_s was determined by Barth *et al.* (2001). The correlation coefficient r for linear regression was between 0.96 and 1.00. Control and water stress treatments were statistically compared. Comparison of means from six separate experiments, each in three replications was done by the Student t – test.

Results and Discussion

The maximum quantum efficiency of PSII photochemistry, estimated by the ratio F_v/F_m (Fig. 1), gradually decreased with increasing the degree of water loss and it was significantly reduced at 20% RWC ($p < 0.001$). Desiccation of *Haberlea* plants at high temperature reduced the photochemical activity of PSII more compared to dehydration at optimal temperature. Exposure of plants to 38 °C lowered the ratio F_v/F_m by 20%. However, the PSII activity quickly recovered after rehydration.

The signal difference $\Delta A_{810\text{max}}$ served as a relative measure for the photochemical capacity of PSI, in the following termed 'potential PSI activity' (Harbinson and Woodward, 1987; Weis and Lechtenberg, 1989). The P700 oxidation rose with increasing the far-red light intensities in *Haberlea* leaves dehydrated to 50% RWC and it was stronger when the dehydration was carried out at 38 °C. The PSI activity was inhibited

when RWC dropped to 10% (Fig. 2).

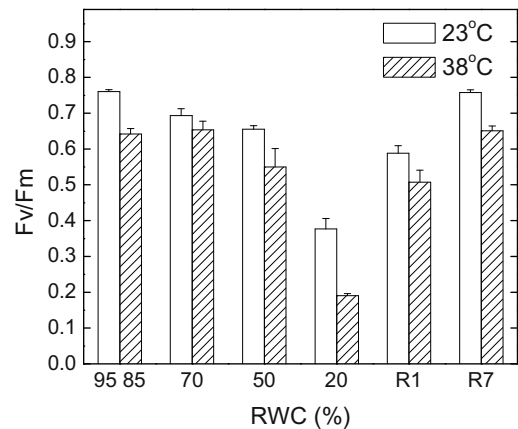


Fig. 1 Changes in maximum quantum efficiency of PSII (F_v/F_m) during dehydration and after 1 day (stage R1) and 7 days (stage R7) of rehydration of *Haberlea rhodopensis* at optimal (23 °C) and high (38 °C) temperature.

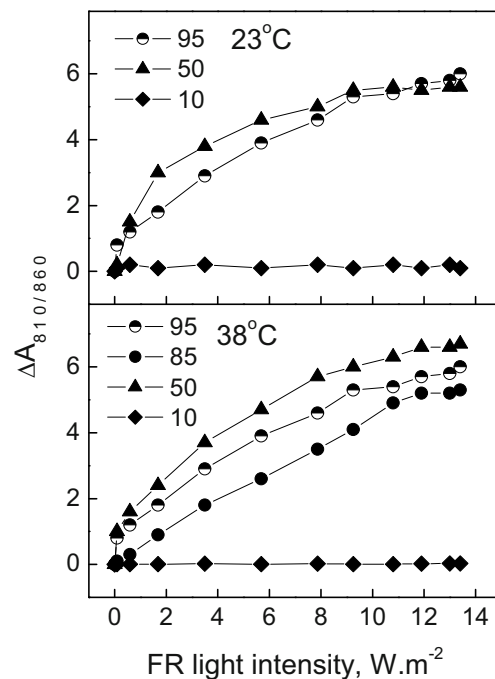


Fig. 2 Changes in the absorption of P700 during dehydration of *Haberlea rhodopensis* at optimal (23 °C) and high (38 °C) temperature.

High temperature treatment did not influence significantly $\Delta A_{810\text{max}}$, but slower enhancement to steady-state levels of A_{810} in light curves was observed (Fig. 2). Therefore saturation constant K_s was calculated from light curves of A_{810} . The values of K_s declined in the course of dehydration but they were higher in plants, treated with high temperature except the plants desiccated to 10% RWC (Fig. 3).

The decrease in K_s observed upon desiccation indicated that lower far-red light intensity was required to oxidize P700. The significantly lower K_s measured after desiccation could be explained by smaller LHCI cross-section and/or lower rate of charge recombination reactions between oxidized P700 and reduced acceptors (Barth *et al.*, 2001) in desiccated state. The results observed could also reflect alterations in optical properties of the desiccated leaves. The investigation of leaf absorbance in the far red region (ΔA_{810}) excited by far red light (> 715 nm) reflected P700 oxidation because PSII was not activated by FR light and linear electron transport in thylakoid membranes of chloroplasts was not induced. Light scattering in leaves is largely determined by the intercellular air spaces (Evans *et al.*, 2004). Scattering is determined by changes in refractive index between air and cytoplasm of the cells. Leaf desiccation caused a wilting of the leaves and reduced volume of intercellular spaces that influenced the leaf absorption properties. Consequently, desiccation of leaves led to changes in the mesophyll tissues inducing a decrease in light scattering and hence a decrease in probability for light capture by P700, *i.e.* decreased far red leaf absorption.

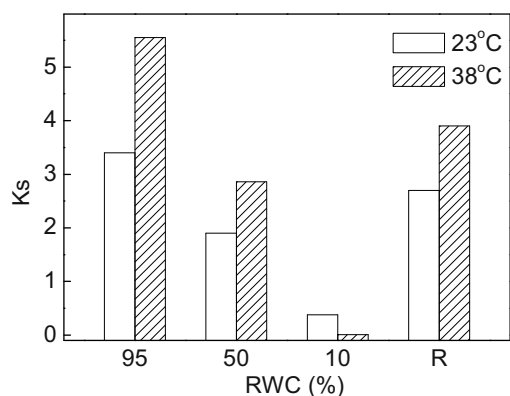


Fig. 3 Changes in the saturation constant of P700, K_s , during dehydration of *Haberlea* plants at optimal (23 °C) and high (38 °C) temperature.

On the other hand, higher values of K_s in high temperature treated plants reflected accelerated PSI activity at such conditions.

The decline in PSII and PSI activity in severely desiccated leaves could represent a protective mechanism from toxic oxygen production in order to maintain the membrane integrity and to ensure

protoplast survival (Havaux and Davaud, 1994; Di Blasi *et al.*, 1998). Additionally, the decline observed in F_v/F_m can be rather a regulatory adjustment to limiting carbon availability, imposed by water stress (Saccardy *et al.*, 1998).

The results showed that desiccation of *Haberlea rhodopensis* at high temperature had more limiting effects than desiccation at optimal temperature. However, the damage was limited to a level where repair was still possible and thus plants fully recovered after rehydration.

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References

- Barth C, Krause G, Winter K (2001) Responses of Photosystem I Compared with Photosystem II to High-Light Stress in Tropical Shade and Sun Leaves. *Plant, Cell and Environ.* 24: 163-176
- Berry JA, Björkman O (1980) Photosynthetic Response and Adaptation to Temperature in Higher Plants. *Annu. Rev. Plant Physiol.* 31: 491-543
- Di Blasi S, Puliga S, Losi L, Vazzana C (1998) S. Stapfianus and E. Curvula cv. Consol in Vivo Photosynthesis, PSII Activity and ABA Content during Dehydration. *Plant Growth Regul.* 25: 97-104
- Evans J, Vogelmann T, Williams W, Gorton H (2005) Sunlight Capture; Chloroplast to Leaf. In: Smith W, Vogelmann T, Critchley C (eds.), *Photosynthetic Adaptation: Chloroplast to Landscape*. Springer, pp. 15-41
- Gaff DF (1971) Desiccation Tolerant Flowering Plants in Southern Africa. *Science* 174: 1033-1034
- Harbinson J, Woodward F (1987) The Use of Light-Induced Absorbance Changes at 820 nm to Monitor the Oxidation State of P-700 in Leaves. *Plant, Cell & Environ.* 10: 131-140
- Havaux M, Davaud A (1994) Photoinhibition of Photosynthesis in Chilled Potato Leaves Is Not Correlated with a Loss of Photosystem-II Activity. *Photosynth. Res.* 40: 75-92
- Klughhammer C, Schreiber U (1998) Measuring P700 Absorbance Changes in the Near Infrared Spectral

- Region with a Dual Wavelength Pulse Modulation System. In: G Garab (ed.), *Photosynthesis: Mechanisms and Effects*. v. V, pp. 4357-4360
- Noctor G, Foyer CH (1998) Ascorbate and Glutathione: Keeping Active Oxygen under Control. *Annu Rev. Plant Physiol. Plant Mol. Biol.* 49: 249-279
- Saccardy K, Pineau B, Roche O, Cornic G (1998) Photochemical Efficiency of Photosystem II and Xanthophyll Cycle Components in Zea Mays Leaves Exposed to Water Stress and High Light. *Photosynth. Res.* 56: 57-66
- Tuba Z (1998) Ecophysiological Responses of Homoiochlorophyllous and Poikilochlorophyllous Desiccation Tolerant Plants: a Comparison and an Ecological Perspective. *Plant Growth Regul.* 24: 211-217
- Tyystjarvi E, Karunen J (1990) A Microcomputer Program and Fast Analog to Digital Converter Card for the Analysis of Fluorescence Induction Transients. *Photosynth. Res.* 26: 27-132
- Weis E, Lechtenberg D (1989) Fluorescence Analysis during Steady-State Photosynthesis. *Philosophical Transactions of the Royal Society, London B* 323: 253-268