

LIGHT DEPENDENCE OF PHOTOSYNTHETIC OXYGEN EVOLUTION OF *HABERLEA RHODOPENSIS* DESICCATED AT HIGH TEMPERATURE

Mihailova G., S. Petkova, D. Stefanov, K. Georgieva*

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

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Summary. The effect of light on the rate of oxygen evolution of *Haberlea* leaves desiccated at optimal (23°C) and high (38°C) temperatures was investigated. The maximum rate of photosynthesis (A_{\max}) decreased in the course of desiccation and this effect was stronger at high temperature. Activation of alternative electron sinks (AES) was found in *Haberlea* plants desiccated to 50% RWC at high temperature as compared to those desiccated at optimal temperature, as well as after their rehydration. Due to more active dark respiration light compensation was reached more slowly in plants desiccated at high temperature. The enhancement of AES together with increased dark respiration provided energy necessary for the reparatory processes which was important for overcoming the stress.

Key words: drought, high temperature, light curves, oxygen evolution, resurrection plants.

Abbreviations: AES – alternative electron sink, HDT plants – homoiochlorophyllous desiccation tolerant plants, LCP – light compensation point, PDT plants – poikilochlorophyllous desiccation tolerant plants, R – recovery, RWC – relative water content.

INTRODUCTION

Among vascular plants, 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 plants (HDT), which retain their chlorophyll during drying and poikilochlorophyllous plants (PDT), which lose chlorophyll on

*Corresponding author: gkm_rw@abv.bg

drying (Tuba, 1998). The photosynthetic apparatus is very sensitive and liable to injury, and needs to be maintained or quickly repaired upon rehydration (Ramanjulu and Bartels, 2002). Water deficit leads to a closure of stomata together with a decrease in the intercellular CO₂ concentration. Because of the lower CO₂ availability, carbon assimilation is inhibited and ultimately photosynthetic capacity is lost. In conditions of water deficit, stomata close can limit the supply of CO₂ to chloroplasts. This event inhibits carbon fixation and causes overexcitation of chlorophylls, which then transfer their energy to oxygen, giving rise to singlet oxygen and further production of reactive oxygen species (Kranner and Lutzoni, 1999; Navari-Izzo and Rascio, 1999). The protection against desiccation damages in angiosperms includes the production of nonreducing di- and oligosaccharides, various compatible solutes and specific proteins, such as the late embryogenesis abundant proteins and heat shock proteins as well as some changes in lipid composition (Hoekstra et al., 2001). The loss of chlorophylls and the breakdown of the chloroplast inner structure in PDT plants, leaf folding and chlorophyll shading together with the production of antioxidants in HDT plants are very important to prevent light-associated damage during desiccation (Sherwin and Farrant, 1998). Under natural conditions drought is often associated with high temperature and high irradiance, 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 of the enzymes involved in photosynthetic carbon metabolism (Berry and Björkman,

1980). *Haberlea rhodopensis* Friv. is an endemic species descending from tropic-subtropic family of *Gesneriaceae* survived as a tertiary relict on the Balkan Peninsula. 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 this study was to investigate the effect of light on the photosynthetic activity of *Haberlea* leaves desiccated at optimal (23°C) and high (38°C) temperatures.

MATERIAL AND METHODS

Plant material, desiccation and rehydration

Plants were subjected to drought stress by withholding irrigation either at 23/20°C or 38/30°C day/night temperature, an irradiance of 30 μmol m⁻² s⁻¹ PPFD, a 12/12 h photoperiod, and relative humidity 60%. After desiccation to air-dry state, the plants were rehydrated in a reconstructed exsiccator providing permanent humidity by a water pump. Control plants, kept at 23/20°C or 38/30°C were regularly watered during the experiment. The measurements were conducted at different degree of dehydration (RWC of 70, 50 and 8%) as well as after 24 h of rehydration.

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 calculated by the formula:

$$\text{RWC (\%)} = \frac{(m_{\text{fresh}} - m_{\text{dry}}) \times 100}{(m_{\text{saturated}} - m_{\text{dry}})} \quad (1)$$

Oxygen evolution

Oxygen evolution rate was determined using a leaf disk electrode (Type LD2/2, Hansatech, UK). It was measured at 50, 120, 350, 500 and 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD at saturating CO_2 concentration (provided by a carbonate/bicarbonate buffer).

The dependence of the rate of photosynthesis A on the light intensity was modelled by a nonrectangular hyperbola (Ögren, 1993):

$$\theta A^2 - (\Phi_{\text{O}_2} I + A_{\text{max}}) A + \Phi_{\text{O}_2} I A_{\text{max}} = 0 \quad (2)$$

where θ is the convexity of the light curve, Φ_{O_2} is quantum yield for O_2 evolution and A_{max} is the light-saturated rate of O_2 evolution.

Alternative electron sinks (AES) were calculated following Ribeiro et al. (2003):

$$\text{AES} = (\Delta F/\text{Fm}')/\Phi_{\text{O}_2} \quad (3)$$

Statistics

Control and water stress treatments were statistically compared. Comparison of means from six separate experiments, each in three replications was done by the Student's t -test.

RESULTS AND DISCUSSION

Haberlea rhodopensis, growing under low irradiance in its natural habitat, was desiccated to an air-dry state at a similar light intensity (about 30 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD) under optimal (23/20°C) or high (38/30°C) temperatures. Dehydration of plants at a high temperature increased the rate of water loss 3-fold. RWC of the

leaves reached 8% after 24 days or 7 days of drought at 23°C and 38°C, respectively. The light response curves of photosynthetic O_2 evolution of *Haberlea* desiccated to a different extent at 23°C and 38°C are presented in Fig. 1. The values for O_2 evolution increased with increasing the light intensity and it was light saturated at 500-600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD. However, this enhancement of photosynthetic activity with increasing the light intensity was less expressed when decreasing the RWC of the leaves. Exposure of plants to 38°C for a week decreased the rate of O_2 evolution and its value measured at a light intensity of 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD was lower by 13% than the control (Fig. 1B). The results showed that the photosynthetic activity was very sensitive to the drought stress. Measurements of O_2 evolution at 23°C and 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD showed that it decreased by 56% when RWC dropped to 50% and it was fully inhibited at 8% RWC (Fig. 1A). Desiccation of plants at a high temperature reduced further the rate of O_2 evolution at all light intensities. The value of this parameter measured at 70 and 50% RWC and 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD was by about 30% lower compared to the plants desiccated to the same RWC at 23°C. Moreover, following rehydration the recovery of the photosynthetic activity of plants desiccated at 38°C was slower compared to plants desiccated at 23°C (Fig. 1). The light dependence of O_2 evolution rate was modelled by a nonrectangular hyperbola (Ögren, 1993). The results are presented in Fig. 2. The maximum value for oxygen evolution, estimated from the light curve (A_{max}) decreased in the course of desiccation and it was stronger in plants desiccated at higher temperature. As it was described above the recovery

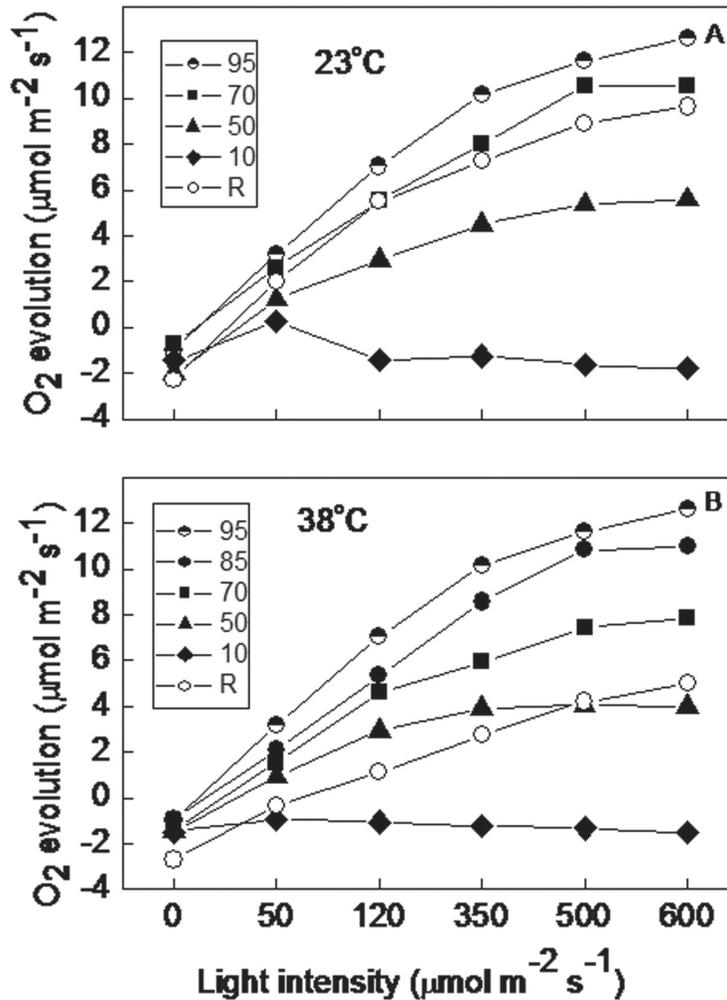


Fig. 1. The light response curves of photosynthetic O₂ evolution of *Haberlea rhodopensis* desiccated to a different extent at 23°C (A) and 38°C (B).

of the latter was slower. The initial slope of light curve of oxygen evolution, Φ_{O_2} , reflects the quantum efficiency of O₂ evolution. The observed higher reduction of A_{max} of plants desiccated to 70% RWC at 38°C than that at 23°C did not correlate with the changes of Φ_{O_2} . Thus, it could be suggested that the observed decline of A_{max} could be due to some regulative processes rather than to injury (Ribeiro et al., 2003). The results on AES (alternative electron sinks, including the cyclic electron flow around PSI and PSII and

chloroplast respiration) revealed that the regulative processes were not related with the increased cyclic electron transport (Fig. 2). However, activation of AES was found in *Haberlea* plants desiccated to 50% RWC at a high temperature, as well as after their rehydration. An enhancement of AES provides ATP necessary for reparation processes. The results on light compensation point (LCP) showed that due to more active respiration light compensation was reached slower in plants desiccated at a high temperature.

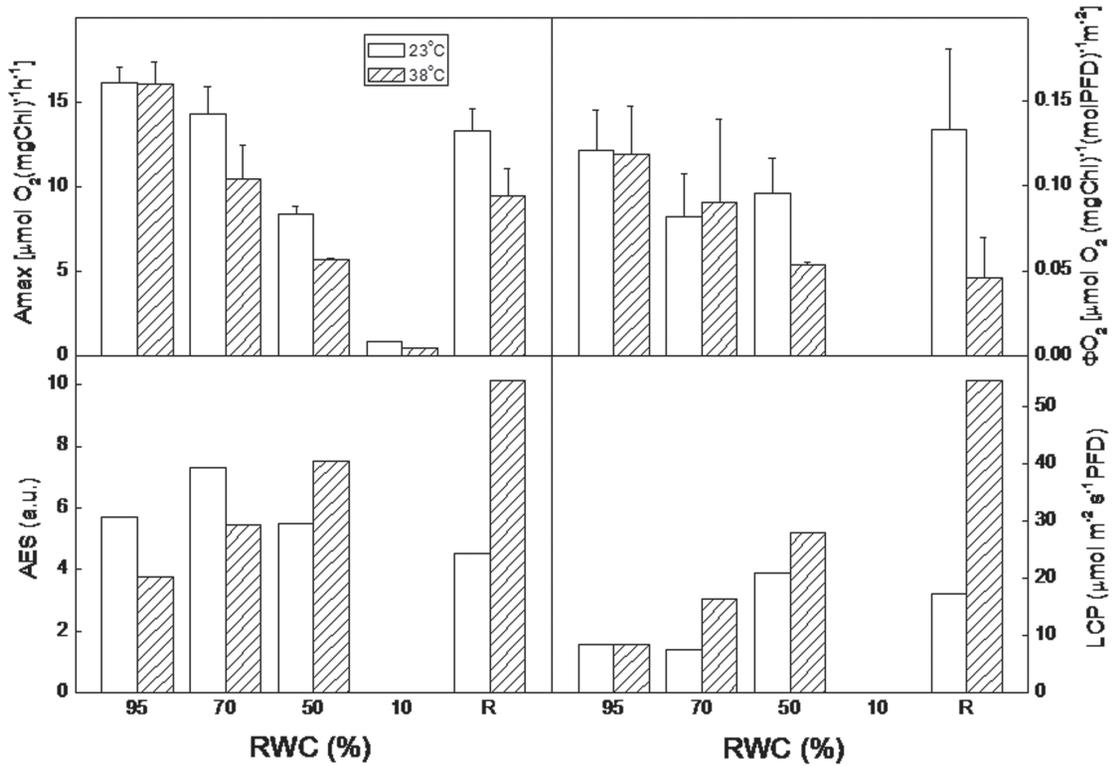


Fig. 2. Modeled oxygen evolution parameters of *Haberlea* plants desiccated to a different extent at 23°C and 38°C calculated by eq. 2. For more details see Materials and Methods.

The values for LCP after rehydration of plants dried at 23°C and especially of those dried at 38°C were much higher than the control, indicating that dark respiration was prevailing over photosynthesis. Both AES and dark respiration acceleration could be considered as important protective mechanisms since on the one hand, the production of active oxygen species, which are produced mainly as a result of photosynthesis, could be limited and on the other, respiration provides energy for the reparation processes.

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