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Gas exchange and malate accumulation in *Haberlea rhodopensis* grown under different irradiances

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Abstract

Diurnal patterns of CO_2 exchange and fluctuations of tissue malic acid concentrations were investigated in the resurrection angiosperm *Haberlea rhodopensis* Friv. grown under irradiances of 30 or 300 μmol(photon) m^{-2} s^{-1} at transition from biosis to anabiosis and *vice versa*. Different degree of CAM-cycling were exhibited under well-watered conditions and extreme desiccation under both irradiances. The CAM-cycling was proved as efficient mechanism of saving water.

Additional key words: anabiosis, CAM-cycling, Crassulacean acid metabolism, resurrection plant.

Introduction

The Crassulacean Acid Metabolism (CAM) is usually regarded as a metabolic adaptation of very specialized plants to long-term drought periods (Kluge and Ting 1978). Recently, it has become clear that CAM is widespread among plants from very different habitats, including submerged water plants (Keely 1996) and covers large diversity of gas exchange patterns (Griffiths 1988, Smith and Winter 1996). These plants optimize their carbon metabolism and gas exchange very flexibly in response to changes in environment. Thus, the mode of photosynthesis can be changed reversibly and quickly from approximately that of a C_3 plant to an exclusive night-time CO_2 uptake within few days with or without greatly affecting nocturnal accumulation of malic acid (Winter and Smith 1996).

One of the species showing a high plasticity of CAM during the transitions from biosis to anabiosis and *vice versa* is resurrection angiosperm *Haberlea rhodopensis* Friv. (Markovska *et al.* 1997). This poikilohydrous endemic species descending from the tropic-subtropic family of *Gesneriaceae* survived as a tertiary relict on the Balkan

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Abbreviations: Δmal - accumulated malate; E - transpiration rate; HI - high irradiance; LI - low irradiance; P_N - net photosynthetic rate; WSD - water saturation deficit; WUE - water use efficiency.

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Peninsula. Recently, it occurs as a typical chasmophyte which inhabits sun exposed or shaded, northern, chiefly limestone slopes up to an altitude of more than 1800 m. Its natural habitats are characterized by high mean annual temperatures (about 20 °C), frequent mist and dew fall in the early morning hours and in the evening, and high periodic rainfalls (annual mean 600 mm). Besides episodic droughts and high temperatures, pronounced short- and long-term changes in irradiance may be the major environmental constraints affecting gas exchange pattern.

The possibility of the operation of CAM as a means of tolerating drought, and the observation that the plants occupy both shaded and fully exposed areas led us to studying the functioning of CAM, and photosynthetic response to extreme desiccation and following recovery to full turgidity in plants grown in climate chambers with two contrasting irradiances.

Materials and methods

Plants and growth conditions: Whole plants were collected from the same locality of Batchkovo Monastery in May during the phase of flowering. The plants collected were stored in an anabiotic state at room temperature and relative humidity (~55 %) under dim light for 12 months. The recovery of the metabolic activity of the plants was carried as described by Markowska *et al.* (1994). The tufts were planted in a mixture of forest soil and gravel and watered to full soil moisture capacity.

Further, the plants were watered every 3 d with one-third of the initial quantity of water. Six months prior to experiments the pots with the plants were transferred to a climate chambers with a 12-h photoperiod, day/night temperature 32/28 °C, relative humidity 60/65 % at two irradiances: high irradiance (HI; 260 - 300 μmol m^{-2} s^{-1}), and low irradiance (LI; 20 - 30 μmol m^{-2} s^{-1}). The growing conditions in the chambers corresponded to those in the natural habitats of the plants. The plants were watered daily for at least five months before drought was imposed by cessation of watering.

The studies were carried out with control plants in which watering continued during the whole experimental period, plants droughted for one month (transition from biosis to anabiosis), and with plants rehydrated for 8 d (transition from anabiosis to biosis).

Net photosynthetic (P_N) and transpiration (E) rates were measured with a portable photosynthetic system *LI-6000* (*LI-COR*, Lincoln, USA). For each experiment, 8 - 10 mature leaves were used whose water saturation deficit (WSD) was determined gravimetrically immediately after measurement. The studies were accomplished in three consecutive years (1996 - 1998). P_N and E were integrated for 12 h to obtain values for both the light and dark periods. Water use efficiency (WUE) was calculated as P_N/E.

L-Malate concentration was determined enzymatically (Gutmann and Wahlefeld 1974). Accumulated malate (Δmal) was calculated as the difference between values at the beginning and the end of the photoperiod. CO_2 recycling was calculated indirectly as the difference between Δmal and the integrated night-time P_N (Borland and Griffiths 1989). The amount of water potentially saved through recycling of respiratory CO_2 was estimated by dividing CO_2 fixed at night by daytime WUE and was expressed as a percentage of daytime transpirational water loss (Martin *et al.* 1988).

Results and discussion

Net photosynthetic rate (Fig. 1*A*,*D*) and malate accumulation (Table 1) in well-watered plants of *H. rhodopensis* corresponded to the CAM-cycling mode which is a variant of CAM (Ting 1985). In plants grown under high irradiance most CO_2 uptake occurred during the light period (Fig. 1*A*). However, in plants grown under low irradiance similar P_N uptake was observed during the light and dark periods (Fig. 1*D*). The malate accumulation, occurring as a result of recycling of respiratory CO_2 (CAM-cycling) was higher in LI plants (Table 1). Irradiance and temperature are known to influence greatly P_N, and acid accumulation of several CAM plant species (Kluge and Ting 1978, Osmond 1978). For many CAM plants, a night temperature between 10 and 20 °C, and high daytime temperature, was optimal for night-time CO_2 uptake and acid accumulation (Neales 1973, Medina *et al.* 1977, Haag-Kerwer *et al.* 1992). High night temperatures decreased night-time acid accumulation because of respiratory loss of accumulated acids (Kaplan *et al.* 1976) or an increased malate efflux from the vacuoles (Friemert *et al.* 1988).

Table 1. Amount of malate accumulated overnight [Δmal, mmol kg^{-1}(d.m.) $(12\ h)^{-1}$], percentage of recycled CO_2, water saved [mol kg^{-1} d^{-1}], and percentage of daytime water loss in *H. rhodopensis* grown under high or low irradiances. See Material and methods for details.

Irradiance	Treatment	Δmal	Recycling	Water saved	[% of water lost]
High	control	50	28.5	1.23	4
	stress	119	84.7	1.80	18
	rehydration	69	1.2	1.92	5
Low	control	86	59.1	2.08	12
	stress	68	87.2	1.06	13
	rehydration	24	0.2	0.81	4

LI plants showed higher conservation of water than HI plants (Table 1). Our findings agree with the suggestion of Martin *et al.* (1988) that CAM-cycling is a much more efficient mechanism of saving water than CAM. Its functioning is of primary importance for the survival of the species during sudden drought, since it is directly associated with their water balance. As drought frequently occur in the *H. rhodopensis* habitat, the creation of a potential reserve of water is the only chance of avoiding lethal water stress and might help in the preservation of physiological activity. WSD in the leaves of the droughted HI and LI plants increased from 26.20 ± 3.2 % to 92.14 ± 1.34 % and from 22.47 ± 1.06 % to 74.01 ± 2.64 %, respectively. In water-stressed plants a strong reduction of P_N during light period was observed (Figs. 1*B*,*E*, 2*A*,*B*). Drought of HI plants resulted in a decrease of integrated CO_2 fixed in the night-time, but quantity of malate accumulated overnight and the percentage of recycled CO_2 increased. Potential conservation of water increased from 4 to 18 % of total daytime transpiration (Table 1). On the contrary, in the leaves of droughted LI plants, the quantity of malate accumulated overnight as well as potential conservation of water decreased in comparison with control. The percentage of daytime transpirational water loss remains similar.

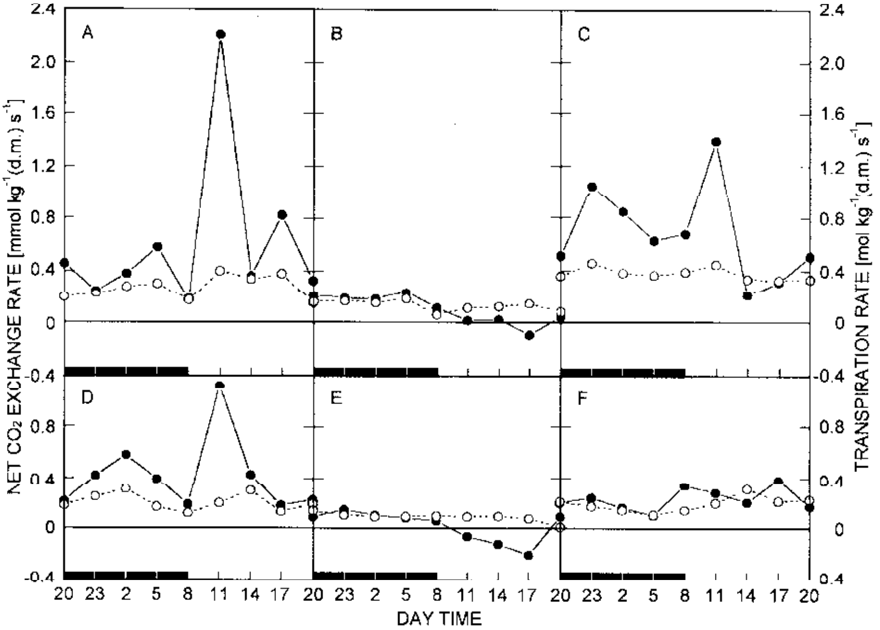

Fig. 1. Diurnal changes in net photosynthetic rate (*closed circles*) and transpiration rate (*open circles*) of *H. rhodopensis* grown under high (*A*,*B*,*C*) or low (*D*,*E*,*F*) irradiances. Comparison of control plants (*A*,*D*), water stressed plants (*B*,*E*) and rehydrated plants (*C*,*F*). Means of 8 - 10 replicates. SE lower than 10 %. The *solid bars* on the x-axes indicate the period of darkness.

WSD in the leaves of the rewatered HI and LI plants was 23.64 ± 6.63 % and 20.01 + 4.63 %, respectively. The share of night-time P_N (Figs.1*C*, 2*A*) and E (Figs. 1*C*, 2*C*) remained higher in rehydrated HI. WUE in night time was higher during drought and recovery compared that of the control HI plants (Fig. 2*E*). The amount of water saved in percentage of daytime water loss in HI and LI rehydrated plants was lower than in droughted plants (Table 1).

H. rhodopensis belongs to the homoiochlorophyllous desiccation tolerant group which during water stress retain their chlorophyll content above 80 % (Markovska *et al.* 1994) and their photosynthetic apparatus remained essentially unchanged. In poikilochlorophyllous desiccation tolerant group of plants drought results in the loss of chlorophylls (Hambler 1961, Gaff 1977, 1989, Tuba *et al.* 1994).

On the 8th day of rehydration of *H. rhodopensis*, the malate accumulated overnight as well as water saved in the tissues of HI plants were higher than in control (Table 1). However, our preliminary investigations showed that CO_2 fixation did not recommence until the 8th day (Markovska *et al* 1997), although the chloroplast ultrastructure completely recovered as early as 48 h after the onset of rewatering (Markovska *et al.* 1995). The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase and, above all, of phosphoenolpyruvate carboxylase increased not earlier than on the 8th day (Markovska and Kimenov 1998).

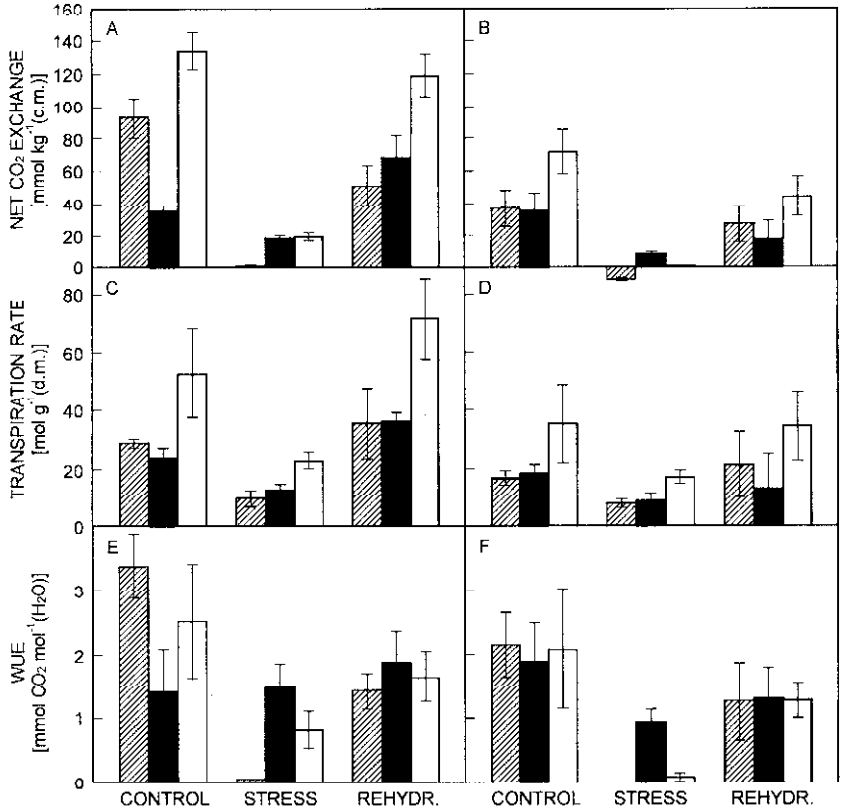

Fig. 2. Integrated CO_2 uptake (*A,B*) or water loss by transpiration (*C,D*), and WUE (*E,F*) during light period (*hatched columns*), dark period (*closed columns*), and whole 24-h period (*open columns*) in *H. rhodopensis* grown under high (*A,C,E*) or low (*B,D,F*) irradiances. Comparison of control plants, water stressed plants, and rehydrated plants. *Vertical bars* denote SE, $n = 24 - 30$.

The reason for the high flexibility of CAM-cycling, leading to relatively fast growth under HI, is probably due to the utilisation of the moisture remaining on the rocks in the early morning and late night hours when the mist falls. The higher percentage of potentially saved water in the tissues in anabiotic state favours the rapid restoration of the vital functions after rewatering. At LI, CAM-cycling serves only for keeping the growth and slower transition from biosis to anabiosis. On desiccation, the percentage of potentially saved water in the plant tissues is approximately twofold smaller than in the control plants. That is why the restoration of the vital functions under LI is slower.

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