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Regulation of CAM and Respiratory Recycling by Water Supply in Higher Poikilohydric Plants – *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panč. at Transition from Biosis to Anabiosis and Vice Versa

Y. Markovska¹, T. Tsonev², and G. Kimenov¹

¹ University of Sofia, Faculty of Biology, Department of Plant Physiology, Sofia, Bulgaria

² Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bontchev, Sofia, Bulgaria

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Abstract: In this paper the expression of C₃ and CAM in the resurrection plants *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panč. during the transition from biosis to anabiosis and vice versa is reported for the first time. The transition from predominantly C₃ metabolism to net dark fixation of CO₂ occurred in leaves of *R. serbica* during desiccation. Desiccated plants of *H. rhodopensis* react by reducing light assimilation of CO₂. When watering was resumed night time fixation of CO₂ by *R. serbica* was observed within 24 hours. The recovery of CO₂ fixation by *H. rhodopensis* was not seen until the 8th day. Desiccated and rehydrated plants of *H. rhodopensis* recapture a higher proportion of respiratory CO₂ than well-watered plants. Since both species have little capacity for water conservation in their tissues, the early onset of high recycling of CO₂ following drought could be an important mechanism for potentially saving water.

Key words: Higher poikilohydrides, drought, recovery, Crassulacean Acid Metabolism, carbon recycling, water saving.

Introduction

The Balkan endemic plants – *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panč. (Gesneriaceae) encountered in the Bulgarian flora, belong to the group of higher desiccation-tolerant or "resurrection" plants. They are tertiary paleophytic relicts with a narrow and chance distribution due to the combination of anabiotic drought resistance with hygromorphic anatomical structure. The first attribute aids in surviving drought and overheating, and the second favours normal functioning only in localities with ample atmospheric humidity. Both species grow exclusively on the sparse soil layer of northerly-exposed rock outcrops below the forest region. Under these conditions, they are protected against the direct action of the sun. The lack of rain during the vegetative period, however, results in rapid desiccation and transition into the anabiotic state. Our preliminary investigations show induction of Crassulacean Acid Metabolism (CAM) in the leaves of both plants after 21 days of water stress (Markovska et al., 1989). The occurrence of CAM was previously studied in four epiphytic species of the Gesneriaceae by Guralnick et al. (1986).

The aim of the present study is to verify the flexibility of the CO₂ fixation pathway in view of adaptation of the photosynthetic apparatus to extreme desiccation and the changes following recovery. The presence of C₃ photosynthesis, CAM and recycling of respiratory CO₂ are shown to be influenced by plant water status.

Materials and Methods

Plant material

The plants were collected from the same locality in May during flowering. The habitat of *R. serbica* is close to an open water basin which creates higher relative air humidity and small temperature variations. *H. rhodopensis* withers completely by the end of July, while the vegetation period of *R. serbica* continues till the end of August. The plants collected were stored in an anabiotic state at room temperature and relative air humidity (~55%) under dim light for a period of 12 months. In this way the material was made uniform with respect to water and temperature. The recovery of metabolic activity began after planting the tufts in a mixture of forest soil and gravel and watering to full water capacity of the soil. Further, the plants were watered every 3 days with one-third of the initial quantity of water. The pots with the plants were transferred to a climatic chamber with a 12 h photoperiod (from 8.00 to 20.00 h), temperature 32/28 °C, relative air humidity 60/65% and photon flux density (PPF) 65 μmol m⁻² s⁻¹, measured at the upper leaf surface. The transition from metabolic activity (biosis) to anabiosis was followed after adaptation of the plants to the new conditions for 4–6 weeks without further water.

The studies used water-saturated (control) plants which were watered throughout the whole experiment; plants droughted for 4 and 14 days (transition from biosis to anabiosis); and with plants rewatered on the first and eighth day to full turgidity (transition from anabiosis to biosis). Leaves were collected for determination of carbon isotope ratios from the same locality in April (the beginning of the vegetative period); in May and September (the end of the vegetative period).

Gas exchange measurements

Net CO₂ exchange rate (P_N), transpiration rate (E) and stomatal resistance (r_s) were determined with a portable photosynthetic system LI-6000 (Li-Cor, Lincoln, NE, USA) at PFD as

described in "Materials and Methods". For each variant of the experiment, 6–8 average-aged leaves were used whose water saturated deficit (WD) was determined immediately after measurement by the weight method. The studies were accomplished in three consecutive years (1991–1993). Assimilation and transpiration curves were integrated for 12 h to obtain values for both the light and dark periods. Water use efficiency (WUE) was calculated from the integrated values.

Malate and recycling of CO₂

L-Malate was determined enzymatically after Gutmann and Wahlefeld (1974). Accumulated malate (Δmal) was calculated as the difference between dawn and dusk values. Following Borland and Griffiths (1989), CO₂ recycling was calculated indirectly as the difference between Δmal and the integrated night time net CO₂ uptake ($\text{int } J_{\text{CO}_2}$):

$$\text{recycled CO}_2 = \Delta\text{mal} - \text{int } J_{\text{CO}_2}$$

$$\% \text{recycling} = \frac{\Delta\text{mal} - \text{int } J_{\text{CO}_2}}{\Delta\text{mal}} \times 100$$

The amount of water potentially saved through recycling of respiratory CO₂ was estimated by dividing the recycled CO₂ by net CO₂ assimilation of the plants following Fetene and Lüttge (1991).

Carbon isotope ratios ($\delta^{13}\text{C}$)

Leaf samples for each species ($n=3-5$) were oven-dried at 70°C and ground to a fine powder. The ratios were expressed relative to the Pee Dee Belemnite (PDB) standard, using a procedure similar to that described by Ehleringer and Osmond (1989).

Stereological measurement of internal leaf structure

For each variant of the experiments 5 average-aged leaves were selected and at least 3–5 segments 3 mm long and 3 mm wide were cut from the middle part of the leaf lamina. The samples were fixed in 3% glutaraldehyde, post-fixed in 2% OsO₄ and embedded in Spurr epoxy resin (1969). The ultra-thin sections 1 μm thick were stained for 1 min with 0.5% Toluidine Blue O (Feder and O'Brien, 1968). In order to determine the relative percentage of leaf volume (A) occupied by the total leaf airspace and mesophyll cell volume (A^{mes}), a randomly distributed point grid was used according to Parkhurst (1982). The quantitative measurements were conducted with a Laboval microscope (Karl Zeiss, Jena). The results were processed by variation-statistical methods using the Student's t -criterion (Plohinisky, 1981).

Results

The results of the diurnal course of net CO₂ exchange in control plants of *H. rhodopensis* and *R. serbica* showed that CO₂ fixation takes place both in the day and in the night (Fig. 1 A-a and 2 A-a). They react to desiccation and recovery in different ways. Desiccation of *H. rhodopensis* leads to net CO₂ exchange reduction predominantly during the day (Fig. 1 A-b) and increased water deficit, restricts CO₂ uptake to the early morning (Fig. 1 A-c). In *R. serbica* the increase in

water deficit results in an increase in night time CO₂ fixation (Fig. 2 A-c).

Carbon isotope ratios of plants collected in May and September from their native habitat varied between –26‰ and –25.7‰ for *H. rhodopensis*, and between –22.5‰ and –18.0‰ for *R. serbica*. Both field measurements of $\delta^{13}\text{C}$ and climatic chamber gas exchange studies for *R. serbica* showed a shift from C₃ photosynthesis to CAM at the onset of water stress. Values of $\delta^{13}\text{C}$ for *H. rhodopensis* were characteristic of C₃ plants or of species which perform CAM only in the recycling mode (Martin and Zee, 1983).

On rewatering of *H. rhodopensis*, CO₂ fixation does recommence until the 8th day (Fig. 1 A-e) and is restricted to late night and early morning hours.

On rewatering of *R. serbica*, nocturnal CO₂ fixation is restored within 24 h (Fig. 2 A-d). The decrease in the water deficit leads to an increase in this CO₂ fixation (Fig. 2 A-e).

Values for $\delta^{13}\text{C}$ in *H. rhodopensis* and *R. serbica* in April were –29.4‰ and –16.5‰. These results showed that from the beginning of the vegetative period (April) to May, a shift from CAM to CAM-cycling in *R. serbica* takes place. The occurrence of low values of $\delta^{13}\text{C}$ in *H. rhodopensis* may imply that carbon gain is mainly due to C₃ photosynthesis, with the contribution from CAM being small.

In all variations of the experiments, stomatal resistance (Fig. 1 C and Fig. 2 C) changes inversely proportional to the established relationships of net CO₂ exchange in *H. rhodopensis* (Fig. 1 A) and *R. serbica* (Fig. 2 A).

On the basis of the calculated integrated fixed CO₂ in daytime, night time and the whole 24 hour period (Fig. 3a–Fig. 4a), as well as the quantity of the transpirational water for the same hours (Fig. 3b–Fig. 4b), it is possible to estimate the WUE for the different experiments (Fig. 3c–Fig. 4c). In the control plants of *H. rhodopensis* WUE is considerably higher than in *R. serbica*, for both night and day. The 14 day desiccation produces an increase in diurnal WUE in *R. serbica* compared to the control (Fig. 4c–bar 3). On rewatering WUE in *R. serbica* only becomes noticeable during the night of the 8th day (Fig. 4c–bar 5).

The quantity of malate accumulated during the night is higher in control *H. rhodopensis* than in *R. serbica*, as is the integrated CO₂ fixed during the same period at the expense of the higher percentage of recycled respiratory CO₂ (Fig. 3d–bar 1 and Fig. 4d–bar 1). The 14 day drought of *R. serbica* results in a reduction in accumulated malate and recycled CO₂ in comparison with the control. The plants of *R. serbica* desiccate completely in 21 days when diurnal changes in net CO₂ exchange and transpiration are almost zero, but the fluctuations in the malate remain around 5 mmol g⁻¹ DW 12 h⁻¹. With increasing drought the ratio A^{mes}/A in both treatment increases, and the intracellular space diminishes (Table 1). The differences are statistically significant at higher water deficits on the 14th day of desiccation. The same holds true for changes in leaf thickness.

On rewatering the quantity of accumulated malate in *R. serbica* increases as the water deficit decreases (Fig. 4d–bar 5). The

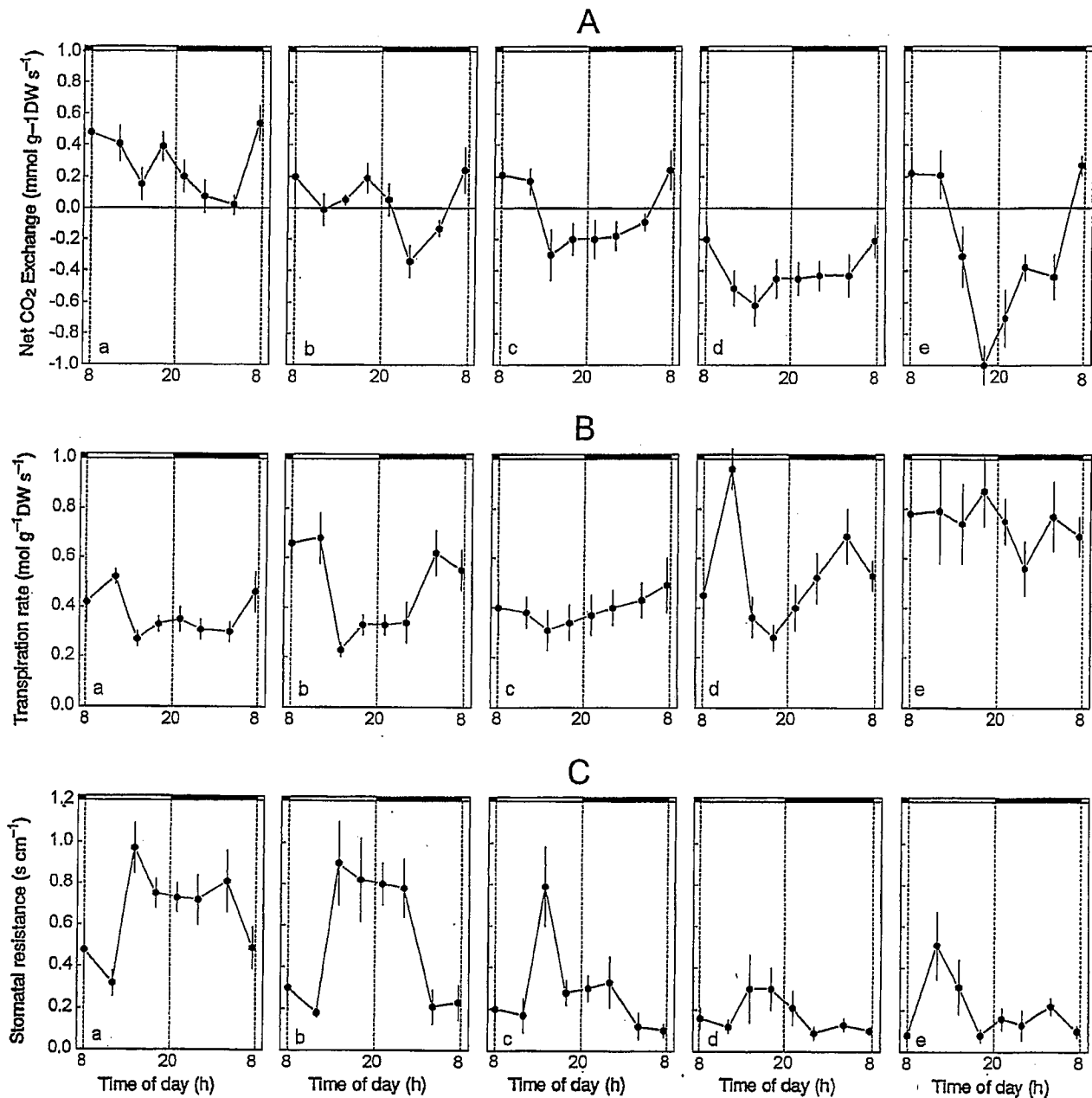


Fig. 1 Changes in the net CO₂ exchange (A), transpiration rate (B) and stomatal resistance (C) in diurnal course of *H. rhodopensis*: a) control variant (WD = 20–25%); b) drought for 4 days (WD = 45–50%); c) drought for 14 days (WD = 85–90%); d) on the 1st day of recovery (WD = 45–50%); e) on the 8th day of recovery (WD = 20–

25%); Each point is the mean of 18–24 observations \pm SE. Positive values of CO₂ exchange indicate net CO₂ uptake, negative values indicate net CO₂ loss. The dark bar on the top indicates the duration of the dark period.

quantity of CO₂ fixed on the 8th day after rewatering is less than that in the control (Fig. 4d–bar 5). The established differences between A^{mes}/A in recovered plants and in control plants are statistically significant only during the first day after rewatering (Table 1). The leaf thickness, however, remains smaller.

Recovered plants of *H. rhodopensis* (as well as plants drying to various high water deficits) capture greater portions of respiratory CO₂ compared to control plants (Fig. 3d–bars

2, 3, 4, 5). For this reason, despite integrated values of nocturnal CO₂ fixation being negative in all experiments, different quantities of malate are accumulated. These are much lower than those calculated for the control plants of *H. rhodopensis*. The differences between A^{mes}/A in recovered plants on the 1st and 8th day and the control plants are statistically insignificant (Table 1).

According to the formula suggested by Fetene and Lüttge (1991), the amount of water potentially saved changes pro-

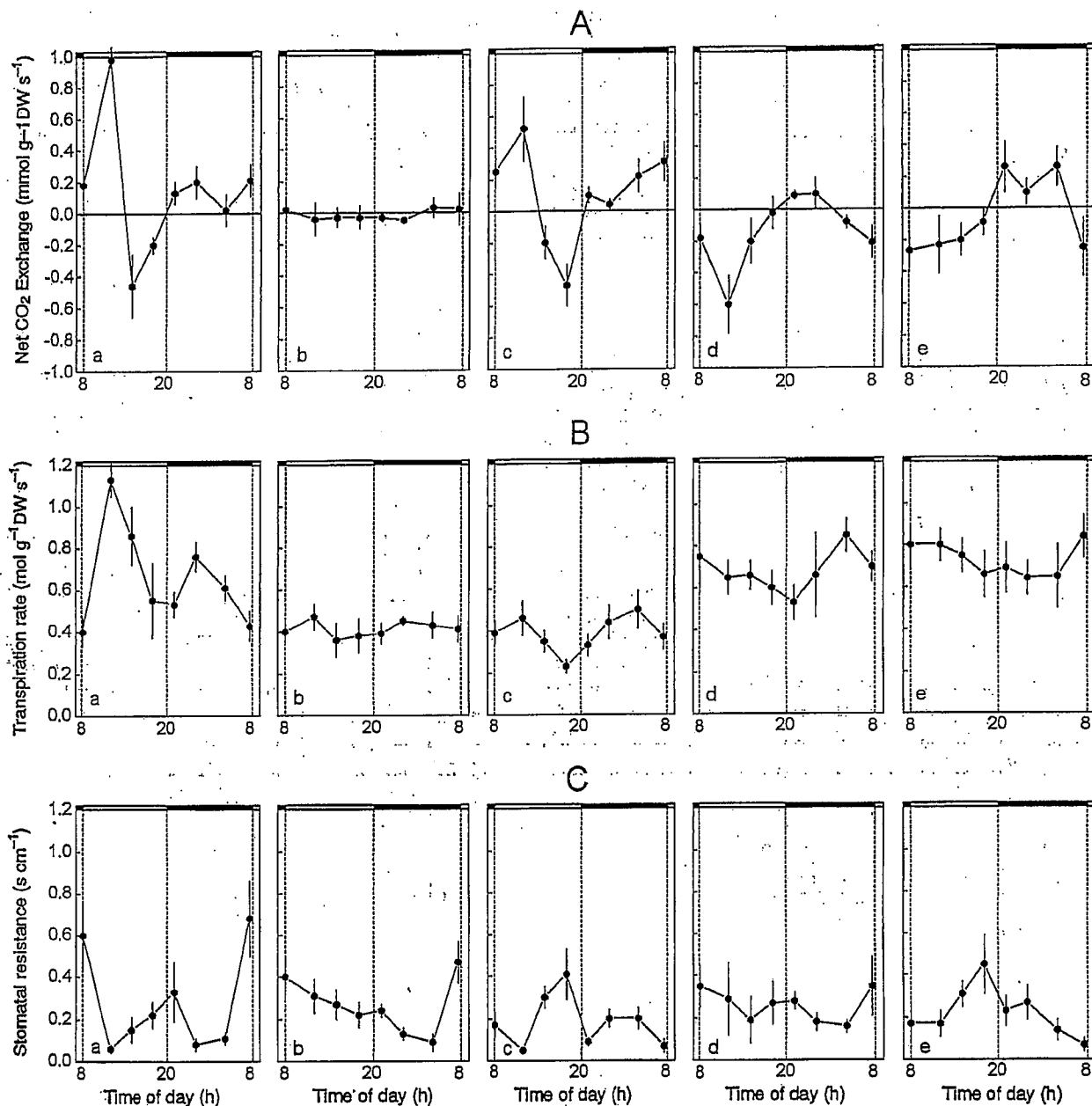


Fig. 2 Changes in the net CO₂ exchange (A), transpiration rate (B) and stomatal resistance (C) in diurnal course of *R. serbica*: a) control variant (WD=20–25%); b) drought for 4 days (WD=45–50%);

c) drought for 14 days (WD=75–80%); d) on the 1st day of recovery (WD=25–30%); e) on the 8th day of recovery (WD=20–25%).

portional to the quantity of recycled respiratory CO₂ in the different treatments (Table 2). The control plants of *H. rhodopensis* are characterized by a higher quantity of recycled CO₂ in comparison with *R. serbica*, and the amount of water potentially saved in their tissues is greater.

Discussion

Photosynthetic gas exchange and malate accumulation in well-watered plants of *H. rhodopensis* and *R. serbica* correspond to the CAM-cycling mode which is a variant of CAM. CAM-cycling is a widespread phenomenon, having been

described in plants from 15 families (Martin et al., 1988), the majority of which are succulents. These plants grow predominantly in thin soil or in crevices of rock outcrops, where periodic drought induces rapid transition from C₃ to CAM (Borland and Griffiths, 1990; Harris and Martin, 1991a, b; Herrera et al., 1991; Martin et al., 1988a, b). An essential role in this transition is played by the recycled CO₂ liberated in respiration. According to Griffiths (1988, 1989); Griffiths et al. (1986) and Martin et al. (1988), recycling of respiratory CO₂ is common to all CAM plants. Direct correlations between recycling and water stress have been reported by Nobel et al. (1978) and Martin and Adams (1987). Our results support

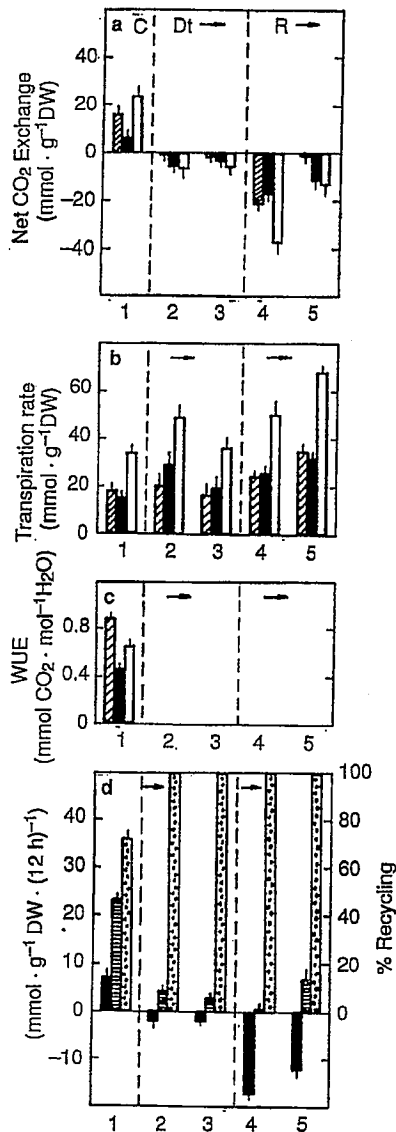


Fig. 3 a) Integrated total net CO_2 exchange during daytime (hatched bars), nighttime (dark bars) and 24-hour period (open bars) by *H. rhodopensis*: 1 - control variant; 2, 3 - on drought for 4 and 14 days; 4, 5 - recovery on the 1st and 8th day; b) integrated transpiration rate during daytime, nighttime and 24-hour period; c) WUE during daytime, nighttime and 24-hour period; d) integrated total CO_2 fixed overnight (dark bars), quantity of malate, accumulated at the same period (horizontal hatched bars) and percentage of recycled CO_2 , released in respiration (dotted bars). The arrows indicate the direction of drought (Dt) and rewatering (R).

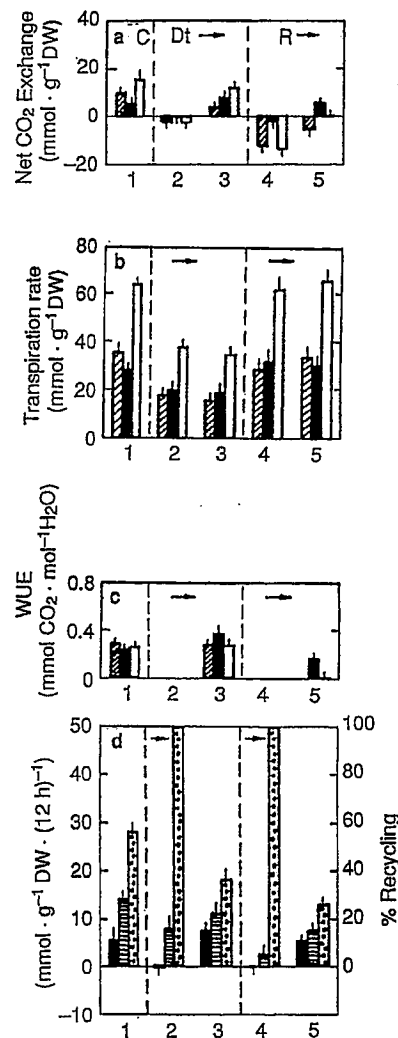


Fig. 4 a) Integrated total net CO_2 exchange during daytime (hatched bars), nighttime (dark bars) and 24-hour period (open bars) by *R. serbica*: 1 - control variant; 2, 3 - on drought for 4 and 14 days; 4, 5 - recovery on the 1st and 8th day; b) integrated transpiration rate during daytime, nighttime and 24-hour period; c) WUE during daytime, nighttime and 24-hour period; d) integrated total CO_2 fixed overnight (dark bars), quantity of malate, accumulated for the same period (horizontal hatched bars) and percentage of recycled CO_2 , released in respiration (dotted bars).

Cockburn's suggestion (1985) of water conservation in plant tissues through recycling of respiratory CO_2 . The ecophysiological importance of this relationship in *H. rhodopensis* (Fig. 3c-bar 1), whose habitat is far from water sources, is essential for its survival. The creation of a potential reserve of water is the only chance of avoiding drought and could aid in the preservation of physiological activity for a longer time. On interruption of watering, a strong reduction of light CO_2 assimilation was observed (Fig. 3a-bar 2,3). This coincides with the gradual decrease in the activity of RuBP carboxylase - the initial carboxylating enzyme in C_3 photosynthesis. Nevertheless, a certain amount of malate is accumulated in the dark period during desiccation owing to recycling of respiratory CO_2 (Fig. 3d-bars 2,3). The activity of PEP carboxylase, responsible for malate synthesis, increases about fourfold by the 4th day after cessation of watering, diminishing gradually afterwards (unpublished data). Minimum physiological activity was preserved up to the 14th day in desiccated plants only in the late night and early morning

hours. This probably coincides with dew fall and the possibility for plant leaves to assimilate additional moisture during.

Thanks to the higher relative air humidity and the weaker temperature variations of its natural habitat, drought in *R. serbica* takes place gradually. The amount of water potentially saved during CAM-cycling in fresh plants was nearly half that of *H. rhodopensis* (Table 2). Fourteen day drought stimulated CAM activity and CO_2 fixed overnight increases (Fig. 4a-bar 3). This improves WUE during the same period (Fig. 4c-bar 3). Our preliminary investigations show that the activity of PEP carboxylase remains high. However, the quantity of recycled respiratory CO_2 diminishes compared to the control, as does the quantity of accumulated malate (Fig. 4d-bar 3). The amount of water potentially saved during CAM decreased by about 30% compared to the control (Table 2). Our results agree with those of Martin et al. (1988) that CAM-cycling is a much more efficient mechanism of saving water and CO_2 than CAM.

Variant of the experiment	Water deficit (%)	Leaf thickness (μm)	Total airspace (%)	A^{mes}/A (%)
<i>Haberlea rhodopensis</i>				
Control	20–25	496 \pm 25 ^a	42.6 \pm 8.2 ^a	55.3 \pm 8.2 ^a
Drought				
– on the 4th day	45–50	442 \pm 23 ^a	35.6 \pm 1.7 ^a	64.3 \pm 7.9 ^a
– on the 14th day	85–90	314 \pm 47 ^b	23.4 \pm 5.6 ^b	77.0 \pm 9.0 ^b
Recovery				
– on the 1st day	45–50	379 \pm 22 ^b	39.7 \pm 4.3 ^a	63.2 \pm 2.8 ^a
– on the 8th day	20–25	443 \pm 09 ^b	37.4 \pm 2.0 ^a	54.0 \pm 5.7 ^a
<i>Ramonda serbica</i>				
Control	20–25	317 \pm 12 ^a	24.2 \pm 4.2 ^a	76.7 \pm 3.3 ^a
Drought				
– on the 4th day	45–50	276 \pm 12 ^b	16.9 \pm 3.2 ^{a,b}	81.8 \pm 4.4 ^a
– on the 14th day	75–80	270 \pm 09 ^b	15.1 \pm 2.3 ^b	85.8 \pm 1.6 ^b
Recovery				
– on the 1st day	25–30	221 \pm 14 ^b	15.2 \pm 1.0 ^b	85.8 \pm 1.8 ^b
– on the 8th day	20–25	263 \pm 17 ^b	22.6 \pm 5.0 ^a	78.3 \pm 1.8 ^a

Table 1 Changes in leaf thickness and leaf volume occupied by total airspace and mesophyll cell volume on droughting and recovery of *H. rhodopensis* and *R. serbica*. Within each column, numbers followed by the same letter are not statistically different at $\alpha = 0.05$ ($n = 45 - 50 \pm \text{S.E.}$).

Table 2 Potential amount of water saved by CO_2 recycling.

Experiment	Quantity of recycled respiratory CO_2 (Amalate-int J_{CO_2})	Water potentially saved by CO_2 recycling
<i>H. rhodopensis</i> (control)	16.51	0.71
<i>R. serbica</i> (control)	8.11	0.49
<i>R. serbica</i> (14 days drought)	4.24	0.36
<i>R. serbica</i> (8 days recovery)	1.88	0.22

The increase in nocturnal fixation of CO_2 with drought improves the carbon and water balance and delays complete desiccation of *R. serbica* and the anabiotic state (21 days against 14 days in *H. rhodopensis*). Under natural conditions the vegetative period of *R. serbica* is prolonged by one month.

CAM-cycling, C_3 and CAM are precursors of CAM-idling (Ting, 1985). Its ecophysiological significance lies in the fact that it maintains plant tissues in an active metabolic state by minimum recycling of respiratory CO_2 . This makes possible the rapid recovery of photosynthetic gas exchange after rainfall.

On rewatering of *H. rhodopensis*, the quantity of accumulated malate increases, owing to the 100% recycling of respiratory CO_2 . However, CO_2 fixation does not recommence until the 8th day (Fig. 1e), although the structure of the chloroplasts completely recovered as early 48 h after the onset of watering (Markovska et al., 1994). The activity of RuBP carboxylase and, above all, of PEP carboxylase, increases not earlier than the 8th day (unpublished data).

On rewatering of *R. serbica* nocturnal CO_2 fixation is resumed within 24 h (Fig. 2d, e) and the activity of PEP carboxylase increases up to the value measured in the control. A decrease in water deficit increases CO_2 fixation and malate in the night (Fig. 4d–bars 4, 5).

H. rhodopensis and *R. serbica* differ in their abilities to avoid drought and to survive and recover after severe desiccation. During the transition of *H. rhodopensis* from biosis to anabiosis night time CO_2 fixation does not increase. The fluctuations in malate decrease immediately after the 4th day of droughting because of fragmentation of the tonoplast membrane (Markovska et al., 1994). During the 14 day drought of *R. serbica* part of the night time CO_2 fixation increases due to the slower loss of turgidity, preservation of the vacuolar compartment, and preservation of the lamellar structure of the chloroplasts. The changes observed in the ultrastructure of the cells on the 14th day of droughting in *H. rhodopensis* do not occur in *R. serbica* until the 21st day (Markovska et al., 1994). The minimum quantity of water stored in the leaves of *H. rhodopensis* in the anabiotic state, compared to *R. serbica*, limits the course of the repair processes. That is why CO_2 fixation is not measurable until the 8 h day after rewatering.

Both plants are characterized by high anabiotic drought resistance, maintained thanks to a number of ultrastructural peculiarities of the cells and metabolic processes which involve the essential role of water. The investigations show that the regulation of recycled respiratory CO_2 by the expression of various intensities of CAM is of vital importance for the maintenance of drought resistance.

References

- Borland, A. M. and Griffiths, H. – The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. J. Exp. Bot. 40 (1989), 57–64.
- Borland, A. M. and Griffiths, H. – The regulation of CAM and respiratory recycling by water supply and light regime in the C_3 -CAM intermediate *Sedum telephium*. Funct. Ecology 4 (1990), 33–39.
- Cockburn, W. – Variation in photosynthetic acid metabolism in vascular plants – CAM and related phenomena. New Phytol. 101 (1985), 3–24.
- Ehleringer, J. R. and Osmond, C. B. – Stable isotopes. In-Plant physiological ecology. Field methods and instrumentation (Eds. by R. W. Pearcy, J. Ehleringer, H. A. Mooney and P. W. Rundel), 281–300, Chapman and Hall, London (1989).

- Fetene, M. and Lüttge, U. – Environmental influences on carbon recycling in a terrestrial-CAM Bromeliad, *Bromelia humilis*. Jacq. J. Exp. Bot. 12 (1991), 25–31.
- Feder, W. and O'Brien, T. P. – Plant microtechnique – some principles and new methods. Amer. J. Bot. 55 (1968), 123–142.
- Griffiths, H. – CAM – a reappraisal of physiological plasticity in form and function. Adv. Bot. Res. 15 (1988), 43–92.
- Griffiths, H. – CO₂ concentrating mechanisms and the evolution of CAM in vascular epiphytes. In – Vascular plants as epiphytes (Ed. by U. Lüttge), vol. 76, 42–86, Ecol. Studies, Springer-Verlag, Berlin (1989).
- Griffiths, H., Lüttge, U., Stimel, K. H., Crook, C. E., Griffiths, N. M., and Smith, J. A. C. – Comparative ecophysiology of CAM and C₃ bromeliads. III. Environmental influences on CO₂ assimilation and transpiration. Plant Cell Env. 9 (1986), 385–393.
- Guralnick, L. J., Ting, I. P., and Lord, E. M. – Crassulacean Acid Metabolism in the Gesneriaceae. Amer. J. Bot. 73 (1986), 336–345.
- Gutmann, J. and Wahlefeld, A. W. – L(-)-Malate. Determination with malic dehydrogenase and DPN. In – Methods in enzymatic analysis (Ed. by H. U. Bergmeyer), 1585–1589, Verlag Chemie, Weinheim (1974).
- Harris, F. S. and Martin, C. E. – Correlation between CAM-cycling and photosynthetic gas exchange in five species of *Tallinum* (Portulacaceae). Plant Physiol. 96 (1991a), 1118–1124.
- Harris, F. S. and Martin, C. E. – Plasticity in the degree of CAM-cycling and its relationship to drought stress in five species of *Tallinum* (Portulacaceae). Oecologia 86 (1991b), 575–584.
- Herrera, A., Delgado, J., and Paraqueatey, I. – Occurrence of inducible CAM in leaves of *Tallinum triangulare* (Portulacaceae). J. Exp. Bot. 42 (1991), 493–499.
- Markovska, Y. K., Kimenov, G. P., and Tsonev, T. D. – Presence of Crassulacean Acid Metabolism in higher poikilohydric plants *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panč. Photosynthetica 23 (1989), 364–367.
- Markovska, Y. K., Tsonev, T. D., Kimenov, G. P., and Tutekova, A. – Physiological changes in higher poikilohydric plants – *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panč. during drought and rewatering at different light regimes. J. Plant Phys. 144 (1994), 100–108.
- Martin, C. E. and Adams, W. W. – CAM, CO₂ recycling and tissue desiccation in the Mexican epiphyte *Tillandsia schiedeana* (Bromeliaceae). Photosynth. Res. 11 (1987), 237–244.
- Martin, C. E., Higley, M., and Wang, W. H. – Ecophysiological significance of CO₂ recycling via CAM in *Tallinum calycinum* Engelm. (Portulacaceae). Plant Physiol. 86 (1988a), 562–568.
- Martin, C. E., Higley, M., and Wang, W. H. – Recycling of CO₂ via CAM in the rock outcrop succulent *Sedum pulchellum* Michx. (Crassulaceae). Photosynth. Res. 18 (1988b), 337–343.
- Martin, C. E. and Zee, A. K. – C₃ photosynthesis and Crassulacean Acid Metabolism in a Kansas rock outcrop succulent, *Tallinum calycinum* Engelm. (Portulacaceae). Plant Physiol. 73 (1983), 718–723.
- Nobel, P. S., Longstreth, D. H., and Hartsock, T. L. – Effect of water stress on the temperature optimum of net CO₂ exchange for two desert species. Physiol. Plant. 44 (1978), 97–101.
- Parkhurst, D. F. – Stereological methods for measuring internal leaf structure variables. Am. J. Bot. 69 (1982), 31–39.
- Plohinskij, N. – Algorithms of biometry, Nauka, Moskva (1981), (in Russian).
- Spurr, A. R. – A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26 (1969), 31–43.

Yu. Markovska

University of Sofia
Faculty of Biology
Dept. of Plant Physiology
D. Tsankov Blvd.
BL-1421 Sofia
Bulgaria

Section Editor: M. Riederer