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Autor: Eller, Benno M. / Ruess, Béatrice R. / Ferrari, Sandro

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Crassulacean acid metabolism (CAM) in the chlorenchyma and hydrenchyma of *Aloe* leaves and *Opuntia cladodes:* Field determinations in an *Aloe*-habitat in southern Africa

Benno M. Eller, Béatrice R. Ruess and Sandro Ferrari

Institut für Pflanzenbiologie, Universität Zürich, Zollikerstraße, 107, CH-8008 Zürich, Schweiz

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Abstract

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The chlorenchyma and the achlorophyllous hydrenchyma of the CAM-plants *Aloe marlothii*, *A. davyana* and *Opuntia ficus-indica* were screened for diurnal oscillations of malate. Samples were taken from adult plants in a habitat in the Rep. of South Africa. Chlorenchymas of all species showed large diurnal oscillations of the malate content whereas hydrenchymas had only low malate contents subject to no or only very small (*A. davyana*) diurnal changes. These small changes could result from a transport of malate from the chlorenchyma to the adjacent layers of the hydrenchyma and vice versa. The results do not support a direct fixation of CO_2 in the achlorophyllous hydrenchyma as proposed by other investigators.

Key words: Crassulacean acid metabolism, CAM, *Aloe, Opuntia*, hydrenchyma, chorenchyma

Introduction

It is generally assumed that the crassulacean acid metabolism (CAM) only occurs in cells where the key processes of CO_2 dark fixation, vacuolar malic acid storage and photosynthesis occur in the same cell (Kluge and Ting 1978). However, many succulents that are able to perform the CAM have an achlorophyllous central hydrenchyma (e.g. Aloe species) or a peripheral water storage tissue without pigmentation, like the multiple epidermis of some species of *Peperomia* (Kaul 1977). In these cases one can assume that nocturnal CO_2 fixation and storage of the organic acids produced (mainly malic acid) are restricted to the chlorenchyma. For *Aloe arborescens* (Kluge et al. 1979) and *Agave deserti* (Smith et al. 1987) no diurnal oscillation of malic acid was found in the central hydrenchyma, and for *Opuntia bigelowii* it was reported (Kluge and Ting 1978) that

external CO_2 was fixed only in the chlorenchyma. However, Earnshaw et al (1987) reported, for *Carpobrotus edulis* and *Senecio madraliscae*, that titratable acidity in the hydrenchyma accounted for approximately 30% of total nocturnal acidification on a per-leaf basis. Since information on the acidification of achlorophyllous hydrenchymas is limited and controverse, field investigations made in southern Africa were used to get some additional information on whether or not diurnal oscillations of the malate content exist in the central achlorophyllous hydrenchyma of small and large Aloes and of *Opuntia ficus-indica*.

Material and methods

The investigations were carried out on adult plants growing in the Sourish Mixed Bushveld vegetation of the Roodeplaat Dam Nature Reserve (van Rooyen, 1993, 1983a) near Pretoria, Rep. of South Africa. The plants involved, the indigenous *Aloe marlothii* Berger and the invader plant *Opuntia ficus-indica* (L.) Mill. grow mainly in the larger bushclumps whereas the indigenous *A. davyana* Schonl. is found at the borders of the bushclumps and also in the open. The amplexicaul, long-deltoid leaves of *A. marlothii* can grow to about 1 m in length, 0.2 m in width and 20 mm in thickness at the base whereas the *A. davyana* leaves are much smaller (about 0.4 m long, 50 mm wide, and 5 mm thick). The Opuntia-cladodes were about 0.25 m long 0.15 m wide and 15 mm thick. Leaves and cladodes had a peripheral chlorenchyma (for *A. marlothii* and *O. ficus-indica* 2.5 to 3.5 mm thick, for *A. davyana* 1.5 to 2 mm thick) and a central hydrenchyma without any pigmentation.

Leaf discs 20 mm in diameter were punched to determine malate concentration and osmolality. The tissues of the discs were separated by cutting between the hydrenchyma and the chlorenchyma. The hydrenchyma-cylinders (about 15 mm long) of *A. marlothii* were cut in the middle to allow measurements of the upper and the lower part of the leaf's hydrenchyma separately. After fresh weight was determined tissue samples were boiled in a vial for 10 min, then crushed and the osmolality of the sap measured using an osmometer (Mod.3B, Vogel, Giessen, FRG). Thereafter, the samples were dried for estimating malate concentrations in the laboratory in Zürich, Switzerland where the water content of the samples was restored by adding an aliquot of distilled water and sonicating for 10 min in a water bath (at 40 °C). In cases where values for osmolality determined on site with fresh samples differed by more than 5% from those determined after rehydration of dried samples in the laboratory it was assumed that the content of osmotically active substances, including malate, had changed between the sampling on site and the determination 2 months later in the laboratory. Consequently, these samples were not used for further determinations. Malate was determined enzymatically after Möllering (1974).

Abbreviation: CAM = Crassulacean acid metabolism.

Results and discussion

Samples were taken during the rainy season assuring a well expressed CAM. All species displayed a substantial nocturnal increase of the malate content in the chlorenchyma (Fig. 1) indicating high CO_2 dark fixation rates, whereas the malate

Fig. 1. Diurnal changes of malic acid content in the chlorenchym (chl.) and the hydrenchym (hydr.) of *Aloe marlothii*, *A. davyana* and *Opuntia ficus-indica*. The figure includes environmental conditions during the sampling at Roodeplaat Nature Reserve on 13/14 January 1987. At about 18H30 a heavy thunderstorm occurred and prevented an accurate determination of temperature and dewpoint temperature of ambient air. Black bar = night.

Opuntia ficus-indica
chl.
hydr.
Malate (mM)
Environmental conditions
Photon irradiance
Air temperature
Dewpoint temperature
Thunderstorm
Temperature (°C)
Photon irradiance (µmol m⁻² s⁻¹)
Aloe mariothii
upper chl.
lower chl.
upper hydr.
lower hydr.
Aloe davyana
Local time (h)
12:00
18:00
0:00
6:00
12:00

content of the achlorophyllous hydrenchymas varied, except for *A. davyana*, only little. Malate, in samples of *A. marlothii*, was estimated separately for the upper (adaxial) and also for the lower (abaxial) chlorenchymas, and the adjacent hydrenchyma. The two chlorenchyma- and the two hydrenchyma-samples of *A. marlothii* had similar diurnal courses of malate concentrations (Fig. 1). One must emphasize that all species investigated had hydrenchymas completely free of pigments that were sharply separated from the chlorenchymas and thus were typical leaf succulents with partially succulent leaves as defined by von Willert et al. (1992). Our determinations in general confirmed the results for the species already investigated which also have partially succulent leaves, such as *Aloe arborescens* (Kluge et al. 1979), *Agave deserti* (Smith et al. 1987) and *Opuntia bigelowii* (Kluge and Ting 1978). The assumption appears to be justified that in this type of leaf-succulents performing CAM, the noctunal CO_2 fixation is restricted to the chlorenchyma where during the light period photosynthesis occurs.

However, there was undoubtedly a distinct but small nocturnal increase in the malate content of the *A. davyana*-hydrenchyma (Fig. 1). This change in malate concentration could either result from a translocation of malate from the chlorenchymas into the adjacent hydrenchyma layers or from CO_2 dark fixation in the hydrenchyma as postulated by Earnshaw et al (1987). If a CO_2 dark fixation in the hydrenchyma increases photosynthesis in the chlorenchyma, then the CO_2 fixed in the hydrenchyma must be released there and enter the chlorenchyma by diffusion, as postulated by Earnshaw et al. (1987) or, the fixed CO_2 must be translocated in the form of malate from the hydrenchyma to the chlorenchyma. Since one half of the *A. davyana*-hydrenchyma is as thick (about 1 to 1.5 mm) as the adjacent chlorenchyma, a translocation of malate from the chlorenchyma to the hydrenchyma (during the dark period) and vice versa (during the light period) seems more feasible than a CO_2 dark fixation in the hydrenchyma itself. However, final conclusions cannot be drawn yet since data on the presence or absence of diurnal oscillations in malate concentration of an achlorophyllous hydrenchyma are still scarce. Further investigations with hydrenchymas cut into thin layers parallel to the chlorenchyma, and the use of radioactively labeled CO_2 could improve information on this topic.

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