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Crassulacean Acid Metabolism and Crassulacean Acid Metabolism Modifications in *Peperomia camptotricha*[1]

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ABSTRACT

***Peperomia camptotricha*, a tropical epiphyte from Mexico, shows variable forms of Crassulacean acid metabolism (CAM). Young leaves exhibit CAM-cycling, while mature leaves show an intermediate type of metabolism, between CAM and CAM-cycling, having approximately the same amount of nighttime gas exchange as daytime. Metabolism of young leaves appears independent of daylength, but mature leaves have a tendency toward more CAM-like metabolism under short days (8 hours). Large differences in the physical appearance of plants were found between those grown under short daylengths and those grown under long daylengths (14 hours). Some anatomical differences were also detected in the leaves. Water stress caused a switch to CAM in young and mature leaves, and as water stress increased, they shifted to CAM-idling.**

Recently, two modifications of CAM have been reported. Typical CAM plants restrict stomatal opening and CO_2 uptake mainly to dark periods and show large diurnal acid fluctuations. A metabolic modification of CAM, called 'CAM-idling,' usually occurs when CAM plants are water stressed and results in stomatal closure, no gas exchange, and a continued but small diurnal fluctuation in organic acids due to recycling of internal CO_2 (7). The second modification, 'CAM-cycling,' is characterized by C_3-type gas exchange combined with diurnal acid fluctuation. Stomata are open and CO_2 is taken up during the day, yet there is diurnal recycling of acids (7).

In some plants, environmental factors play a role in the regulation of photosynthetic carbon metabolism. Water stress has long been known to have a major influence. It may cause an induction of CAM in plants showing C_3 metabolism. For example, *Portulacaria afra* and *Mesembryanthemum crystallinum* show C_3-type metabolism, but when they have low water status, they shift to CAM (11, 13). Some CAM plants as well may be affected by water status, such as cacti. When stressed, they shift to CAM-idling (3, 7). Besides plant water status, several other environmental conditions may have a regulatory role. Temperature and light are important in regulating the gas exchange of the CAM plants *Sempervivum montanum* (14) and *Tillandsia usneoides* (8). CO_2 uptake by *Tillandsia* is also influenced by RH (8). Photoperiod is a factor in the carbon metabolism of *Kalanchoë blossfeldiana* (2). Gas exchange is C_3 under long days, and when daylength is short, CAM is induced.

Different types of carbon metabolism may even be found within the same plant. *Cissus quadrangularis* leaves, for example, have C_3 gas exchange while the stems become increasingly more CAM-like as they mature (12). In general, when a plant exhibits more than one type of carbon metabolism, older leaves are more prone to CAM than younger leaves (7). For example, CAM can first be induced in mature leaves of *M. crystallinum* (13). *K. blossfeldiana* and *K. velutina* carbon metabolism is influenced by leaf age as well as photoperiod (1). In *Bryophyllum fedtschenkoi*, mature leaves exhibit CAM while young leaves are C_3 (6).

This report describes variations in carbon metabolism of *Pe-*

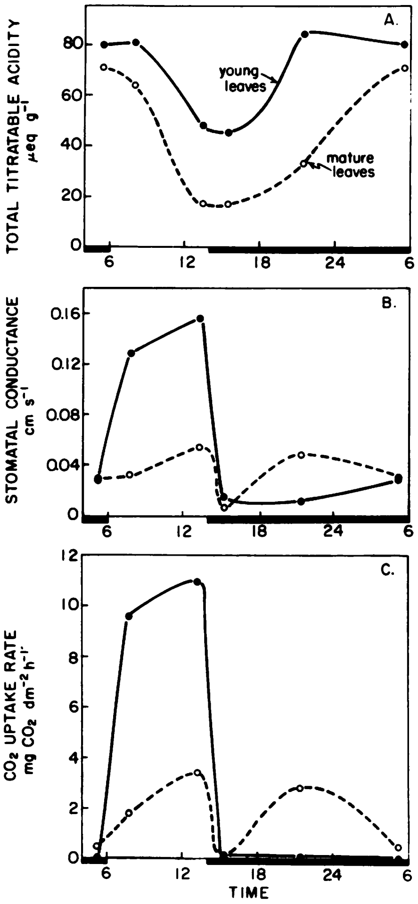

FIG. 1. Diurnal course of organic acids (A), stomatal conductance (B), and rate of CO_2 uptake (C) in young leaves (●——●) and mature leaves (○- - -○) of *P. camptotricha*. This experiment was conducted with an 8-h daylength (the dark period is indicated by the black bar on the abscissa). Daytime conditions were kept at a temperature of 28°C, approximately 50% RH. PAR for young leaves was 500 to 900 $\mu E\ m^{-2}\ s^{-1}$ and for mature leaves 400 to 600 $\mu E\ m^{-2}\ s^{-1}$. At night, temperature was 23°C, RH about 60%, and no light.

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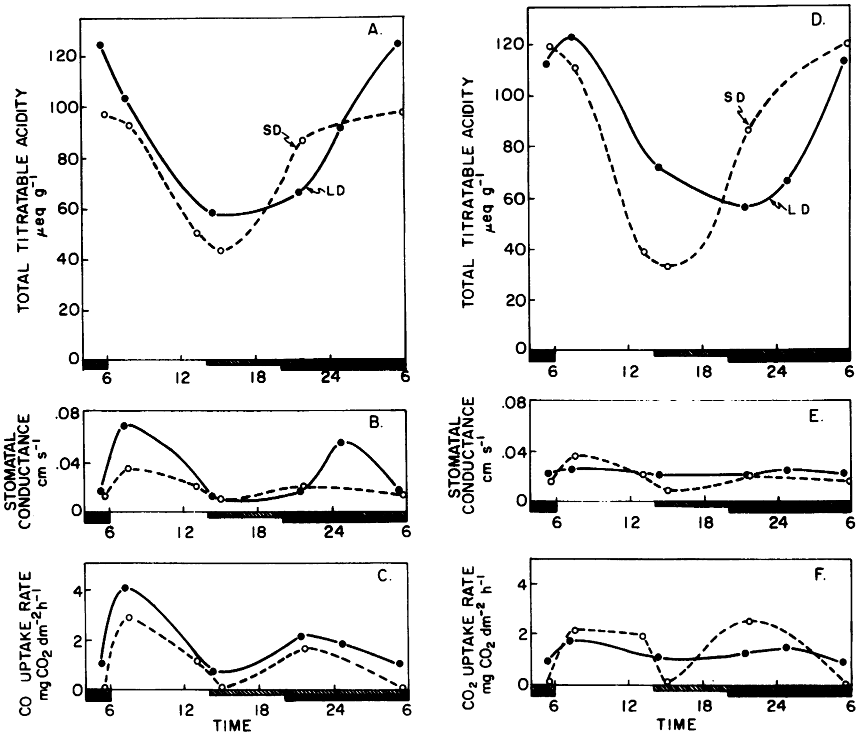

FIG. 2. Diurnal course of organic acids, stomatal conductance, and CO_2 uptake in mature leaves under LD of 14 h (●——●) and SD of 8 h (O- - -O). A–C, at chamber conditions for 2 weeks; D–F, at chamber conditions for 2 months. The black bar indicates the dark period for LD treatment, and the hatched bar indicates the SD dark period. Daytime conditions were kept at a temperature of 28°C, approximately 50% RH, and PAR was 400 to 600 $\mu E\ m^{-2}\ s^{-1}$. At night, temperature was 23°C, RH about 60%, and no light.

FIG. 3. Photographs of *P. camptotricha* after 4 months in growth chambers under SD, 8-h daylength (left) and under LD, 14-h daylength (right).

peromia camptotricha, an epiphytic succulent from southern Mexico. *P. camptotricha* shows CAM-cycling, CAM, CAM-idling, or an intermediary metabolism between CAM-cycling and CAM, as influenced by leaf maturity and environment.

MATERIALS AND METHODS

Plants. *Peperomia camptotricha* plants were propagated by cuttings from a plant grown at the University of California, Berkeley, Botanic Gardens (53.108-1). Cuttings were rooted in sand and later transplanted to 10-cm pots containing sandy loam soil. Plants were grown in a glasshouse and later transferred to growth chambers for experimentation. Plants were kept in Western Environmental growth chambers under defined conditions for at least 1 week prior to analysis of carbon metabolism. Daytime conditions were maintained at a temperature of 28°C, RH approximately 50%, PAR for young leaves was 500 to 900 $\mu E\ m^{-2}\ s^{-1}$ and for mature leaves 400 to 600 $\mu E\ m^{-2}\ s^{-1}$, depending on the position of the leaves. At night, the temperature was 23°C, RH about 60%, and no light. Daylength was either 8 or 14 h. Data were collected on young and mature leaves. Young leaves were in the first 'false whorl' of leaves nearest the apex that were partially developed and only about 1 cm in length. Each 'whorl' usually consisted of five leaves. Mature leaves were taken from the eighth to tenth whorl down from the young leaves. Nonstressed plants were watered every 3 d with a nutrient solution.

Water Stress. Plants were stressed by withholding water for 1 or 2 weeks. In comparison with well-watered controls, stressed plants were markedly wilted. Due to the succulence of the leaves and the significant water storage tissue (multiple epidermis), water potential may not be an accurate indicator of plant water status. Thus, these measurements were not made.

Acid Titrations. Leaves were collected for acid titrations in triplicate and were frozen until assayed. Each sample was ground in a hand tissue grinder with glass-distilled H_2O. Extracts were titrated to an endpoint of pH 7 with 0.01 N KOH using an automatic titrator.

Gas Exchange. CO_2 uptake and stomatal conductance were simultaneously determined using a dual isotope (THO and $^{14}CO_2$) porometer (5). A small chamber was attached to a leaf, and a stream of gas passed across the abaxial surface of the leaf. The gas consisted of 330 $\mu l\ l^{-1}$ $^{14}CO_2$ in a N_2:O_2 mixture of 80:20 which was passed through tritiated water for humidification.

Leaf Anatomy. Leaf tissue was fixed in FAA[2] (4), then rapidly

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[2] Abbreviations: FAA, formaldehyde-acetic acid-alcohol; DMP, 2,2-dimethoxypropane; PAS, periodic acid-Schiff's reagent.

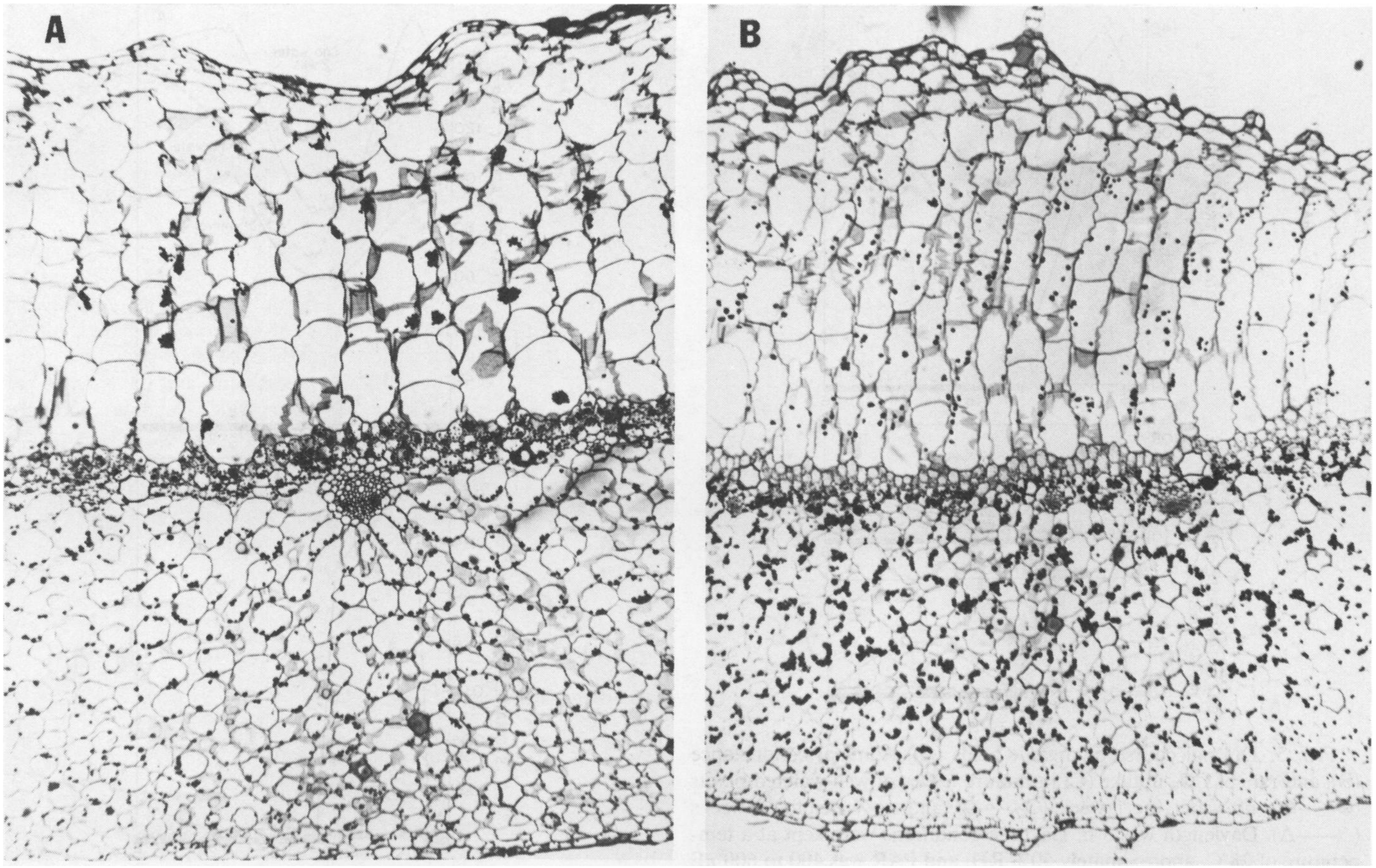

FIG. 4. Cross-sections of older leaves from: A, SD plants; B, LD plants, shown in Figure 3. Note the three tissue layers, multiple epidermis, median palisade, and spongy parenchyma. ×70.

dehydrated in DMP, and stored in 100% acetone. The leaves were embedded in glycol methacrylate and sectioned on a Bright ultramicrotome at 4 μm. Sections were stained with PAS, 20 min in periodic acid and 10 min in Schiff's reagent (9). PAS is a stain for insoluble carbohydrates.

RESULTS

Age of Leaf Tissue. Diurnal levels of organic acids, stomatal conductance, and CO_2 uptake rates were measured in young and mature leaves of *P. camptotricha*. The diurnal activity of younger leaf tissue was different from that of mature leaves, regardless of environmental conditions. Younger leaves had characteristically C_3-type gas exchange (Fig. 1). During the light period, stomata were open and there was net uptake of CO_2, usually in the range of 10 to 12 mg CO_2 dm^{-2} h^{-1}. There was virtually no nighttime uptake of CO_2. Although gas exchange was typical C_3, there was a significant diurnal fluctuation of organic acids, indicative of recycling of internal CO_2. Older leaves, on the other hand, had approximately equal gas exchange day and night. CO_2 uptake rates and stomatal conductance were much less than in the younger leaves. Both young and mature leaves responded to initiation of the dark period by stomatal closure, but older leaves reopened stomata and continued CO_2 uptake throughout the night. Stomata closed once again just prior to the light period. CO_2 uptake rates, day or night, usually varied between 2 and 4 mg CO_2 dm^{-2} h^{-1} for older leaves. These leaves also had a large diurnal fluctuation in acids, equal to or greater than that of the younger leaves.

Daylength. A difference in carbon metabolism was detected between SD and LD plants. These differences developed with time as the plants were kept under constant daylengths of either SD or LD. Plants were taken from the greenhouse and placed in growth chambers with 8- or 14-h daylengths (SD and LD, respectively). There was little variation in the gas exchange patterns of young leaves between the two treatments. Older leaves showed more variability. After only 2 weeks at chamber conditions, little difference could be detected between the carbon metabolism of SD and LD plants (Fig. 2, A–C). Organic acid fluctuation was similar in magnitude and pattern with respect to the daylength. Stomatal conductance was greater in LD plants in the morning and again at night, but conductance was reduced in early afternoon until dark. Stomatal conductance in SD plants was fairly constant throughout the day/night cycle. The patterns of CO_2 uptake were similar in SD and LD plants. After 2 months at chamber conditions, however, SD plants showed a greater acid fluctuation than LD plants (Fig. 2D). Gas exchange in LD plants remained at low levels and was relatively constant over the diurnal period, with a slight depression from midday to dark (Fig. 2, E and F). The gas exchange of SD plants remained about the same as at 2 weeks in the growth chamber, except nighttime CO_2 uptake rates exceeded daytime rates.

Plants kept at chamber conditions for 4 months were developmentally different under short days than under long days (Fig. 3). The LD plants had reduced internodal elongation resulting in a stunted appearance. Leaves were much smaller in area, thinner, and not as turgid as the SD plants; however, stained leaf sections of each treatment show the same basic leaf anatomy (Fig. 4). The leaves of SD and LD plants are made up of three layers with approximately the same number of cells in each comparable layer. The multiple epidermis (water tissue) of the LD plants had smaller and more elongate cells than the SD leaf. The dark green median palisade layer of the SD plant had starch

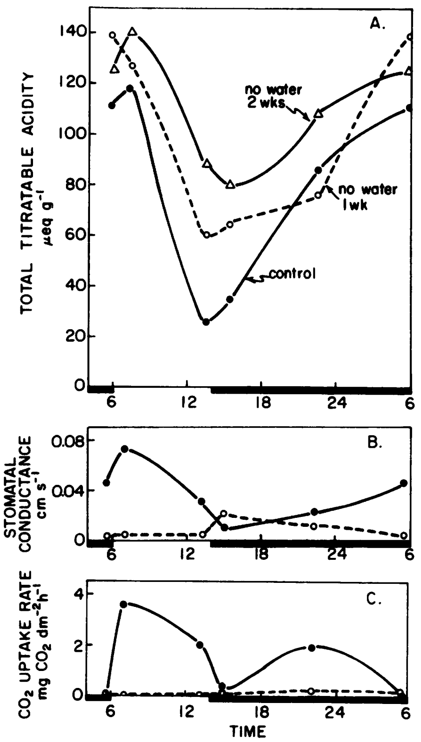

FIG. 5. Diurnal course of organic acids (A), stomatal conductance (B), and rate of CO_2 uptake (C) in mature leaves of well-watered plants (●——●), no water for 1 week (○- - -○), and no water for 2 weeks (△——△). Daylength was 8 h. Daytime conditions were kept at a temperature of 28°C, approximately 50% RH, and PAR was 400 to 600 μE m^{-2} s^{-1}. At night, temperature was 23°C, RH about 60%, and no light.

in all three cell layers, whereas only the bottom cell layer contained starch in the LD leaf. The spongy parenchyma of the SD plant had larger cells than the LD, but contained less starch in that layer.

Water Stress. In young and mature leaves, water stress caused a reduction in carbon metabolism. When water was withheld for 4 d, no difference was detected from those watered the day prior to experimentation. A significant reduction in gas exchange occurred when water was withheld for 1 week, and there was a further reduction at 2 weeks (Figs. 5 and 6). In older leaves (Fig. 5), organic acid fluctuation was dampened with prolonged drought. In the water-stressed plants, gas exchange occurred at night, when compared to the control with significant uptake during the day. Gas exchange data for the plants stressed 2 weeks are not shown in Figure 5 (B and C) because data followed the baseline. There was no detectable CO_2 uptake or conductance,yet there was still acid fluctuation (Fig. 5A). In the younger leaves which typically exhibit CAM-cycling, with all gas exchange during the light period, water stress caused some changes (Fig. 6). There was diurnal organic acid fluctuation in the water-stressed plants with little difference between the 1- and 2-week-stressed plants. Stomatal conductance was greatly reduced in the water-stressed plants, but was about equal day and night, while CO_2 uptake rates, also reduced, were higher at night. Essentially all external CO_2 was taken up during the dark period. There was little gas exchange activity in the 2-week-stressed plants; therefore, data are not shown in Figure 6 (B and C).

DISCUSSION

Leaf maturity is an important factor in the pattern of carbon metabolism exhibited by *P. camptotricha*. Young leaves showed CAM-cycling, with typical C_3 gas exchange and organic acid fluctuations. Older leaves had carbon metabolism intermediate between CAM-cycling and CAM. Gas exchange occurred approximately equally day and night, with maximum CO_2 uptake rates similar to those found in CAM plants. In CAM-cycling, there is daytime CO_2 uptake only, while CAM has mostly nighttime CO_2 uptake. Mature leaves of *P. camptotricha*, however, have gas exchange during both day and night.

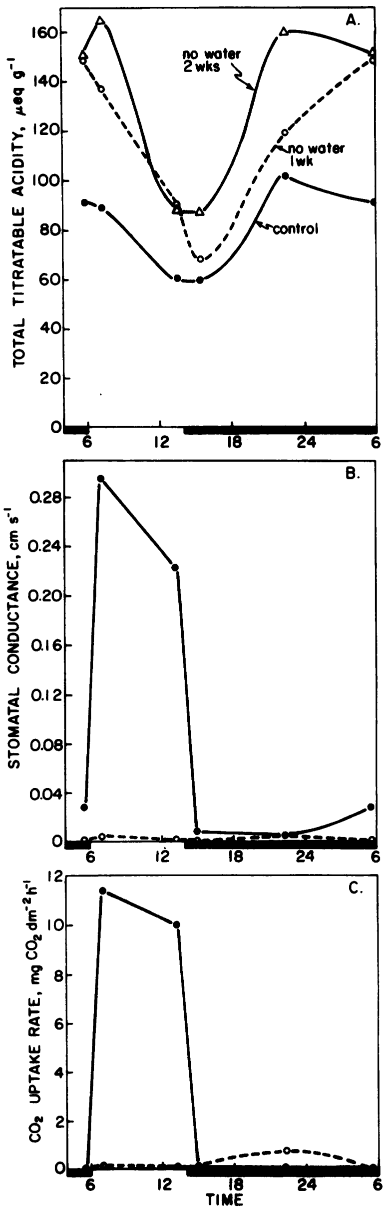

FIG. 6. Diurnal course of organic acids (A), stomatal conductance (B), and rate of CO_2 uptake (C) in young leaves of well-watered plants (●——●), no water for 1 week (○- - -○), and no water for 2 weeks (△——△). Daylength was 8 h. Daytime conditions were kept at a temperature of 28°C, approximately 50% RH, and PAR was 500 to 900 μE m^{-2} s^{-1}. At night, temperature was 23°C, RH about 60%, and no light.

Slight differences in carbon metabolism of mature leaves were observed between SD and LD plants, although to a lesser degree than found in *Kalanchoë blossfeldiana* (1). The period of adaptation to daylength was greater than 2 weeks. This slow response time should be sufficient in the natural environment to regulate seasonal adjustments in carbon metabolism. Short daylength tended to favor slightly more nighttime CO_2 uptake and was accompanied by greater acid fluctuations. Thus, SD plants appeared more CAM-like than LD plants, although both were still intermediary between CAM-cycling and CAM.

Cuttings of the *P. camptotricha* plants studied were initially collected from Mexico at a latitude of approximately 20°. At that location, daylengths range from 11-h days in December to 13.5-h days in June. The winter period, of relatively short days, is generally the driest season with less rainfall. This might result in an adaptation of *P. camptotricha* to be more CAM-like when under SD conditions.

As well as showing variable carbon metabolism due to daylength, plants also showed striking morphological changes in leaf and plant development. The multiple epidermis of leaves of SD plants had larger cells. This would allow for greater water uptake and storage, and could be important to plant survival during periods of infrequent water availability. There could be a division of photosynthetic activity between the three leaf tissue layers suggested by changes in starch localization as influenced by daylength. Also, the spongy parenchyma is morphologically similar to tissue of typical CAM plants. Thus, there may not only be a difference in carbon metabolism among leaves on the same plant, but also perhaps among layers of the same leaf.

Young and mature leaves of *P. camptotricha* responded to water stress by changes in carbon metabolism. With decreasing plant water status, mature leaves went from the CAM-cycling:CAM intermediate to a dampened CAM and finally to CAM-idling. Young leaves that consistently showed CAM-cycling also responded to water stress. As they became progressively more stressed, they proceeded from CAM-cycling to CAM and eventually to CAM-idling. The young leaves, then, did appear to be capable of nighttime CO_2 fixation, at least at low rates. The plants shifted to CAM abruptly between the 4th and 7th d without water. CAM became the dominant pathway and was probably induced by a threshold minimum plant water potential.

Various other environmental conditions were tested, but found to have no detectable affect on photosynthetic carbon metabolism. Some of the factors investigated were different diurnal temperature and humidity regimes, as well as various light intensities.

The carbon isotope ratio of greenhouse-grown *P. camptotricha*, an indicator of photosynthetic pathway, was in the range of C_3 plants, with a value of −27.7 (10). The hydrogen isotope ratio, however, was +23, which is in the CAM plant range. These results support the conclusion made from the gas exchange data that *P. camptotricha* has a form of carbon metabolism intermediate between C_3 and CAM.

P. camptotricha is a good experimental plant for further investigation of the biochemical mechanism and regulation of CAM and CAM modifications, especially CAM-cycling. Within the same plant, different forms of carbon metabolism exist and can be further controlled by environment. The next step will be to explore possible enzymic regulation of the carbon metabolism pathway.

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