Physiological and Isotopic Aspects of Photosynthesis in *Peperomia*

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ABSTRACT
Physiological and isotopic aspects of several *Peperomia* species were investigated. All but one species had C₃-like stomatal behavior, in that stomata were open during the day and closed during the night. In these species, most atmospheric CO₂ uptake occurred during the day. Concurrent with this stomatal behavior, there were Crassulacean acid metabolism-like acid fluctuations in most species. Carbon and hydrogen isotope ratios of cellulose nitrate from *Peperomia* reflect their physiological behavior. The δ¹³C values of cellulose nitrate from *Peperomia* species were similar to values observed in C₄ plants and consistent with the daytime uptake of exogenous CO₂ via the C₃ photosynthetic pathway. The δD values of cellulose nitrate from *Peperomia* species approach those of Crassulacean acid metabolism plants. These elevated δD values are caused by fractionations occurring during biochemical reactions and not as a consequence of water relations.

The phenomenon of CAM is now fairly well understood (12, 26). In typical CAM, gas exchange occurs predominantly at night when stomata are open. Carbon dioxide is taken up at night by carboxylation of PEP¹, catalyzed by the enzyme PEP carboxylase, to form the first product, oxalacetate. Oxalacetate is rapidly reduced to malic acid, which accumulates in vacuoles. During the subsequent light period, malic acid is decarboxylated, and the CO₂ released into the tissue is assimilated through the C₃ photosynthetic cycle. Because stomata are closed, internal CO₂ concentration may increase to over 1% during the day (4). The ecological interpretation of CAM is that it represents a mechanism by which water is conserved, since most gas exchange takes place at night when evaporative demand is low (26).

A modification which has been known for approximately one decade, termed ‘CAM-idling,’ occurs when CAM plants are severely water-stressed and stomata close both day and night. In CAM-idling, there is a continual, but low-rate, cycling of organic acids through the CAM pathway (15, 17, 23, 27). Evidently, the low level of metabolism keeps the plant’s biochemical activity poised until water is available (13, 15–17). Once water is available, the plants recover, usually within 24 h.

Recently, a modification of CAM, termed CAM-cycling, has been described (27). In CAM-cycling, gas exchange occurs largely during the daylight hours, typical of C₃ plants, yet there is diurnal cycling of organic acids in the manner of CAM. CAM-cycling has been observed in *Pereskia* spp. (14), *Cissus quadrangularis* (28), *Welwitschia* (25), *Talinum* (9), the Bromeliaceae (10), and in some species of the Crassulaceae (24).

It is now well known that carbon isotope ratios (¹³C/¹²C) can distinguish C₃ plants from C₄ and CAM plants operating in the CAM mode (1). C₃ plants have ¹³C/¹²C ratios lower than C₄ and CAM plants. It has also been shown that hydrogen isotope ratios (D/H ratios) of cellulose nitrate from CAM plants are much higher than those from C₃ and C₄ plants (19–21). Further, it has been shown that greenhouse-grown CAM-cycling plants have δD values approaching those of CAM plants, although their δ¹³C values are typical of C₃ plants (21).

The purpose of this study was to investigate physiological and isotopic aspects of CAM-cycling as they exist in the genus *Peperomia*. There are at least 300 species of *Peperomia* native to tropical and subtropical regions (7). They are found in South America, in the islands of the Caribbean, southeastern United States, and islands of the Pacific, including Hawaii. Most species are epiphytic and/or lithophytic. The leaf anatomy of these plants is very distinctive, showing an upper hypodermis (multiple epidermis) that may act as a water storage tissue, a median deep green palisade tissue of perhaps three cell layers, and a lower, lighter green, spongy palisade of many cell layers that has the physical appearance of CAM tissue (7).

MATERIALS AND METHODS

**Plant Material.** Isotopic analyses were performed on plants collected at La Selva, Costa Rica. Physiological measurements were conducted on living plants grown in a greenhouse in Riverside, CA. Plants collected at La Selva were propagated from cuttings and grown in the greenhouse. Plants purchased at nurseries or obtained from the University of California Botanic Garden at Berkeley were also grown in the greenhouse. The greenhouse had a mean annual high temperature of 28°C and a mean low of 22°C. Humidity was variable, but in the neighborhood of 40 to 50%. Plants were grown in full glasshouse sun or under shade, depending upon the species. Plants were watered frequently to avoid stress.

**Gas Exchange Studies.** Gas exchange parameters were determined with a dual-isotope porometer (6) on plants from the greenhouse. The porometer passes an air stream of dry **CO₂** (200 µL L⁻¹ in N₂:O₂ mixture of 80:20) through THO of known specific radioactivity. Abaxial leaf surfaces were exposed in triplicate to the radioactive gases for 20 s via a small chamber clamped onto the leaves. The resistances to water vapor transfer and CO₂ uptake (cm s⁻¹) were derived from THO vapor uptake and **CO₂** uptake data that were determined by liquid scintillation counting. Values for conductances (cm s⁻¹) transpiration

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² Abbreviations: PEP, phosphoenolpyruvate; THO, tritiated water; FW, fresh weight.
rates (g water loss dm⁻² h⁻¹), and CO₂ uptake rates (mg CO₂ dm⁻² h⁻¹) were also calculated from the gas exchange data.

**Acid Titrations.** Leaf samples from greenhouse plants were collected, frozen with dry ice, and stored in a freezer until assayed. Individual samples were weighed, ground in glass-distilled H₂O with a motorized Teflon tissue homogenizer, and titrated to a pH 7.0 endpoint with 0.01 N KOH. Data are expressed as μeq acid g⁻¹ FW or as μeq acid cm⁻².

**Isotope Analyses.** Samples of plant material for isotopic analyses were dried in an oven at 50°C, further desiccated in a freeze dryer, and ground to a fine powder in a Wiley mill. cellulose was extracted as previously described (11). Cellulose oxygen isotope ratios were determined by the method of Rittenberg and Ponticorvo (18) as modified by Burkh (3). Carbon and hydrogen isotope ratios of cellulose nitrate prepared from cellulose (11) were determined by a modified version of the Stumpf and Frazer method (11, 22). Isotope ratios are expressed as δ values, where

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

and R represents ¹⁸O/¹⁶O for δ¹⁸O values, D/H for δD values, and ¹³C/¹²C for δ¹³C values. The standards are standard mean ocean water for δ¹⁸O and δD values and the belemnite from the Pee dee formation of South Carolina carbonate for δ¹³C values. The precision of the isotope analyses of cellulose and cellulose nitrate were ±2‰ for δD values, ±0.05‰ for δ¹⁸O values, and ±0.2‰ for δ¹³C values.

**RESULTS AND DISCUSSION**

*Peperomia scandens* is one of the most CAM-like *Peperomia* species that we have tested (Fig. 1). There is a marked diurnal fluctuation of organic acids identical to that of CAM plants. CO₂ fixation shows a definite early morning burst comparable to CAM plants (12), a depression during the day, followed by an increase toward the end of the light period. There is substantial CO₂ fixation at night in comparison to the light period. Also shown in Figure 1 are data for *Peperomia obtusifolia*, one of the least CAM-like of the *Peperomia* species that we have studied. For this species, there is little evidence of dark CO₂ fixation or dark stomatal conductance, even though organic acids tended to be high. We have, however, observed diurnal acid fluctuation for this species previously (6).

Figure 2 shows gas exchange properties for four species of *Peperomia* collected at La Selva and grown in the greenhouse at Riverside. For the most part, CO₂ uptake is typical of C₃ plants, occurring largely during the day. However, in all cases, there appears to be some exogenous CO₂ fixation at night, although rates are rather low. Observations of stomatal conductance expressed in cm s⁻¹ are consistent with CO₂ uptake patterns. Care must be taken in interpretation of the CO₂ fixation data presented here because they were obtained with ¹⁴CO₂ and, thus, net CO₂ fixation was not measured. It is highly likely and indeed probable that the small amount of CO₂ fixation at night, sometimes reaching about 1 mg dm⁻² h⁻¹, is less than the amount of CO₂ lost through respiration. Nevertheless, there appears to be substantial dark CO₂ fixation, since there is a definite, but small, diurnal fluctuation of organic acids similar to that observed in CAM plants, with levels of organic acids reaching values as high as 50 μeq g⁻¹ FW.

We do not have sufficient data to calculate the contribution to acid synthesis by dark CO₂ fixation. However, for *Peperomia* sp. nov., there was no apparent CO₂ uptake at night, yet an organic acid fluctuation of about 15 μeq g⁻¹ FW was measured. Thus, in this species, dark CO₂ fixation seems insufficient to account for acid synthesis.

In summary, the gas exchange and acid flux data (Figs. 1 and
Table II. Carbon and Hydrogen Isotopic Compositions of C3, C4, CAM, and Peperomia spp.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Greenhousea</th>
<th>La Selva, Costa Rica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ13C</td>
<td>δD</td>
</tr>
<tr>
<td>C3</td>
<td>-28.2 ± 1.3^a (10)</td>
<td>-80 ± 35</td>
</tr>
<tr>
<td>C4</td>
<td>-12.6 (2)</td>
<td>-25</td>
</tr>
<tr>
<td>CAM</td>
<td>-15.4 ± 3.2 (21)</td>
<td>+30 ± 19</td>
</tr>
<tr>
<td>Peperomia</td>
<td>-26.0 ± 2.7 (5)</td>
<td>+2 ± 16</td>
</tr>
</tbody>
</table>

*a Previously reported (21).  Data are given as means ± 1 SD.  Numbers in parentheses indicate number of species analyzed.

FIG. 3. a, δD values versus δ13C values of cellulose nitrate from plants collected at La Selva, Costa Rica. The symbols indicate C3 plants (O), C4 plants (□), CAM plants (△), Peperomia species (O), and species from the family Gesneriaceae (▪). Datum points which are underlined with a dashed line are values measured from nonepiphytic terrestrial plants. All other plants sampled were epiphytes. b, δD values of cellulose nitrate versus δ14O values of cellulose for the same plants shown in Figure 3a. SMOW, standard mean ocean water; PDB, belemnite from the Pee Dee formation of South Carolina.

2) indicate that Peperomia spp. may have CAM (P. scandens) or CAM-cycling (all species in Fig. 2), but that some species may show little evidence of these photosynthetic modes (P. obtusi-folia).

Carbon and hydrogen isotope ratios for cellulose nitrate from five species of Peperomia bought at a local nursery and maintained in the greenhouse and nine species of Peperomia collected at La Selva, Costa Rica, are given in Table I. Inspection of the data shown in Table II indicates that δ13C values of Peperomia are comparable to those observed for C3 plants and dissimilar to values of C4 and CAM plants. Cellulose nitrate from Peperomia species is enriched in deuterium relative to that in C3 and C4 plants for both greenhouse and Costa Rican samples, approaching values seen in CAM plants in both cases (Table II).

As shown in Figure 3, a plot of δD values versus δ13C values of samples collected in La Selva allows separation of the Peperomia showing CAM-cycling from C3, C4, and CAM plants. Thus, our previous observations that plants showing CAM-cycling can be identified based on their hydrogen and carbon isotopic compositions are confirmed (21). In addition to Peperomia, Figure 3 displays data for two epiphytes in the Gesneriaceae family (Codana-thane crassifolia and Columnnea linearis), which were also collected at La Selva, Costa Rica. These two plants have the same leaf morphology as Peperomia species. Indeed Codanathane, with the more positive δD value of the two, shows the same carbon metabolism properties as the Peperomia species showing CAM-cycling, whereas Columnnea, with the more negative δD value, has carbon metabolism properties more similar to a typical C3 plant (unpublished observations). Thus, δD values of cellulose nitrate from these two plants reflect the two different types of photosynthetic metabolisms.

The similarity of the δ13C values of the Peperomia species and the C3 plants, sampled either in the greenhouse or at La Selva (Table II), indicates that exogenous carbon fixation in Peperomia is through the C3 pathway. In those Peperomia species showing CAM-cycling, we assume that all CO2 fixed by the CAM pathway at night (when stomata are closed) is the result of refixation of respiratory CO2. This respiratory CO2, coming from carbohydrates synthesized by C3 photosynthesis, would have δ13C values which reflect C3 photosynthesis. Thus, these plants have δ13C values similar to those of C3 plants, and not of CAM plants.

The deuterium enrichment we observed in CAM-cycling Peperomia spp. could be caused by isotopic fractionations occurring during H2O metabolism. Briefly, the enrichment would occur if the leaf water in these plants becomes enriched in deuterium during evapotranspiration and if this H2O then labels the organically bound hydrogen. If this hypothesis is true, cellulose from CAM-cycling plants should also be enriched in 18O relative to C3 and C4 plants, since evapotranspiration enriches leaf water in both D and 18O (2, 5). Measurement of oxygen isotope ratios for the field-grown Costa Rican sample set shows no such oxygen enrichment in CAM-cycling plants (Fig. 3b). In fact, in plants with the various photosynthetic modes, all have similar oxygen isotope ratios. Thus, we conclude that elevated δD values of cellulose nitrate from CAM-cycling plants, like those in CAM plants (19, 20), are due to fractionations occurring during biochemical reactions, perhaps those associated with acid fluctuation.

The contribution of the CAM-cycling phenomenon to the physiology and/or ecology of a plant is not apparent at this time. Peperomia species in their natural environment, being mostly epiphytic, are subjected to frequent periods of drought. Root systems are underdeveloped and thus the plants tend to be hydrated only during and immediately after precipitation. Plants, particularly those species that grow in partial or full sun, become water stressed soon after a precipitation event. We suspect that the CAM-cycling phenomenon is similar to the CAM-idling phenomenon, in which there is organic acid recycling during severe drought when stomata are closed day and night (15, 16). We suggest that drought occurring between precipitation episodes is accompanied by stomatal closure in CAM-cycling plants. If organic acid cyclic continues, the plants would be in the CAM-idling phase and, thus, metabolically poised to resume full photosynthetic activity when water is available. Further investigations are being conducted to ascertain these aspects of CAM-cycling.

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