Selbyana 13: 77–87

THE OCCURRENCE OF CAM IN PEPEROMIA

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ABSTRACT. *Peperomia* is a large pantropical genus of more than 600 species, many of which are epiphytes. A survey indicates that 50% or more of the species have Crassulacean acid metabolism (CAM) or CAM-cycling. Our assessment of 93 taxa is based on the enzymic activities of P-enolpyruvate carboxylase (PEPc) and pyrophosphate: fructose 6-phosphate phosphotransferase (PFP), diurnal organic acid fluctuation, and nocturnal gas exchange and stomatal opening. During development, the leaves of the CAM *Peperomia* spp. shift from C_3-photosynthesis to CAM as evidenced by the appearance of PEPc, the commencement of diurnal organic acid fluctuation, and by a shift from daytime gas exchange to nocturnal gas exchange. There is some evidence that water-stress will accelerate the induction of CAM during development. CAM activity may be seasonal in some species, with more activity during the spring and summer than in the winter when growing in glasshouses. Since the photosynthetic mode of *Peperomia* may change in response to environmental perturbations such as water-stress, changing photoperiods, seasonally, and during development, the assignment of a particular mode to a species may be equivocal. Thus, it is possible that all species have some CAM capacity. The presence of CAM metabolism in this epiphytic genus suggests adaptation to drought and water-stress conditions.

CAM en *Peperomia*

RESUMEN. *Peperomia* es un género pantropical que incluye mas de 600 especies, muchas de las cuales son epífitas. Un estudio indica que el 50% o más de estas especies llevan a cabo metabolismo del ácido crasuláceo (CAM) o CAM-cíclico. Nuestro análisis de 93 grupos taxonómicos está basado en las actividades enzimáticas de P-enolpiruvato carboxilasa (PEPc) y pirofosfato: fructosa 6-fosfato fosfotransferasa (PFP), la fluctuación diurna de ácido orgánico, y la nocturnidad del intercambio de gases y la apertura de los estomas. Durante su desarrollo, las hojas de especies de *Peperomia* de tipo CAM pasan de fotosíntesis C_3 a CAM, de lo cual es evidencia la aparición de PEPc, el comienzo de la fluctuación diurna de ácido orgánico, y el paso del intercambio de gases de diurno a nocturno. Alguna evidencia existe que el stress de agua acelera la inducción de CAM durante el desarrollo. Cuando crecen en invernaderos, la actividad CAM puede ser estacional en algunas especies que son más activas en la primavera y el verano que en el invierno. Debido a que el modo de fotosíntesis de *Peperomia* puede cambiar en respuesta a perturbaciones ambientales como stress de agua y cambios en el fotoperíodo, y con respecto a las estación del ano y la etapa de desarrollo, la asignación de un modo particular a una especie puede ser equívoco. Como cosequencia, es posible que todas las especies tengan cierta capacidad de CAM. La presencia de metabolismo CAM en este género epífito es sugestiva de una adaptación a la sequía y condiciones de stress de agua.

INTRODUCTION

Much recent evidence indicates a substantial number of tropical, flowering epiphytic plants have the photosynthetic metabolism Crassulacean acid metabolism (CAM). Most of these taxa are in the families Bromeliaceae (Coutinho, 1963, 1965; McWilliams, 1970; Medina, 1974; Medina *et al.*, 1977; Griffiths & Smith, 1983), Cactaceae (Kluge & Ting, 1978), Gesneriaceae (Guralnick *et al.*, 1986), Peperomiaceae (Starnecker, 1984; Ting *et al.*, 1985), Rubiaceae (Winter *et al.*, 1983) and Orchidaceae (Coutinho, 1963, 1965; McWilliams, 1970). In addition, many of the hemiepiphytic species of *Clusia* in the Clusiaceae are CAM (Ting *et al.*, 1987). Perhaps the only major groups of flowering plant epiphytes that are not CAM fall within the families Moraceae (*Ficus* spp.) and Araceae (e.g., *Anthurium* spp.) (Ting *et al.*, 1987; Ting, 1989). The contribution of CAM to the productivity of tropical ecosystems would thus be very important (Benzing, 1990).

The predominantly epiphytic *Peperomia* Ruiz and Pavon. (Peperomiaceae) includes species with both C_3 and CAM photosynthesis (Vrizo de Santo *et al.*, 1983; Sternberg *et al.*, 1984; Starnecker, 1984; Ting *et al.*, 1985). Although pantropical, the genus is most diverse in Central and South America, the Caribbean, Hawaii, Polynesia, and the Malay Peninsula (Burger, 1977). Plants are herbaceous with erect, repent, or scandent stems that are rarely over one m tall. All have succulent leaves (Murty, 1960). They are mostly epiphytic but are sometimes terrestrial on rocks or shallow soils. Much of the previous ecophysiological work on *Peperomia* was done by Starnecker (1984), but his work is only reported in an unpublished Ph.D. dissertation. The genus is primitive among dicots and has even been considered to be a monocotyledonous plant (Burger, 1977).

Peperomia is particularly interesting because its diverse photosynthetic mechanisms are accompanied by novel divisions of labor among leaf tissues (Nishio & Ting, 1987). Leaves of *Peperomia* are differentiated into an upper water storing multiple epidermis, a middle palisade parenchyma, a lower CAM-like spongy parenchyma, and a lower single layer of epidermis with the stomata. Although the adaxial multiple epidermis contains about 5% of the chlorophyll (Nishio & Ting, 1987), it lacks stomata and functions primarily in water storage (Kaul, 1977). Because of the presence of the water storing multiple epidermis, the leaves are essentially "window leaves" (Krulick, 1980). The middle one or two cell-layered palisade parenchyma functions in C_3-photosynthesis and contains most of the ribulose bisphosphate carboxylase and chlorophyll (Nishio & Ting, 1987). The lower spongy parenchyma functions in CAM with most of the P-enolpyruvate carboxylase and other enzymes of CAM being present (Nishio & Ting, 1987). Gibeaut and Thomson (1989a, 1989b) studied leaf anatomy of three *Peperomia* species with different degrees of CAM and were able to correlate the thickness of the lower, CAM-like spongy parenchyma in *Peperomia* with the intensity of CAM photosynthesis. However, the taxa they studied may not be representative of the genus, which makes generalizations difficult.

In those species that are CAM, leaves shift from C_3-photosynthesis to CAM during leaf development (Sipes & Ting, 1985; Holthe *et al.,* 1987). Thus, the CAM metabolism in *Peperomia* may be labile. During the normal ontogenetic shift from C_3 to CAM in *Peperomia,* leaves thicken primarily because the CAM-like spongy parenchyma becomes succulent (Holthe, 1988).

CAM is characterized by the presence of high levels of specific enzymes such as P-enolpyruvate carboxylase (PEPc), malate enzyme (ME) and/or P-enolpyruvate carboxykinase (PEPck), and pyrophosphate:fructose 6-phosphate phosphotransferase (PFP) (Ting, 1985; Carnal & Black, 1979, 1983). CAM is also characterized by nocturnal stomatal opening and gas exchange, a massive diurnal fluctuation of malic acid, and a relatively high carbon-13 isotopic composition (Ting, 1985).

In some succulent species, there are high levels of enzymes that are associated with CAM and a diurnal fluctuation of malic acid, but no nocturnal stomatal opening. All exogenous CO_2 uptake is during the day (Ting & Sipes, 1985). Malic acid is synthesized at night from respiratory CO_2 by fixation through the CAM pathway (Patel & Ting, 1987). This latter phenomenon in which malic acid is synthesized at night from respiratory CO_2 when stomata are closed is referred to as CAM-cycling (Ting & Sipes, 1985). In *Peperomia,* species may exhibit C_3-photosynthesis, CAM-cycling, or CAM (Starnecker, 1984; Ting & Sipes, 1985). When severely water-stressed, *Peperomia* species with CAM may shift to CAM-idling characterized by stomatal closure day and night, but with a continued diurnal fluctuation of organic acids (Ting & Sipes, 1985).

In order to determine the extent of C_3-photosynthesis, CAM-cycling, and CAM in *Peperomia,* we have examined gas exchange parameters, organic acid fluctuation, and enzymic activities associated with CAM in 45 different taxa. We have also conducted a literature survey, allowing us to assess a total of 93 taxa.

Materials and Methods

PLANT MATERIAL. Experimental plants were obtained from the University of California Riverside succulent collection, from the University of California Berkeley Botanic Garden, from KDB Custom Farming (Fresno, CA), Glasshouse Works (Stewart, OH), from Ms. Anita Baudean (New Orleans, LA), and from the Department of Botany, Ohio Wesleyan University (Delaware, OH). Plants were propagated from cuttings and grown in a greenhouse in Riverside, CA. Plants in 6-inch pots were irrigated frequently with one-quarter strength Hoagland's solution to preclude nutrient and water stress (Hoagland & Arnon, 1938). Light (PAR) did not exceed 135 μmol m^{-2} sec^{-1}. White-wash was used on the glasshouse to reduce summer radiation and keep the growth houses reasonably light-constant during the year. The mean RH was 35–45% and the mean daytime air temperature was 23–29°C. Night air temperature was kept above 20°C.

GAS EXCHANGE. Gas exchange parameters, CO_2 uptake and stomatal conductance were estimated with a Li-Cor Inc., Model 6000 portable gas exchange system (Li-Cor Inc., Lincoln, NE). A 330 ml polycarbonate chamber with recirculating fans was used as the leaf chamber cuvette. Data were recorded every 6 sec for 1 min to obtain each reading. Each datum is the average of three readings taken from three different leaves of the same plant (Ting *et al.,* 1987).

ORGANIC ACID TITRATIONS Samples from three leaves were collected in triplicate, quickly frozen, and stored on dry ice until assayed. Individual samples were weighed and then ground in glass-distilled water using a coaxial tissue homogenizer with a motor driven Teflon pestle (Potter-Elvehjem). The resulting homogenate was titrated to pH 7.0 with 0.01 N KOH using an automatic titrator. Data are expressed on leaf area and fresh weight basis.

ENZYME EXTRACTION. For enzymatic survey, three leaves were harvested in the early afternoon when leaf acid levels were low. The petiole and mid-rib were removed. Leaf discs with a total area of 12.1 cm^2 were excised from the leaves with a cork borer and ground with a chilled mortar and pestle in 5 ml of ice-cold buffer following a protocol modified from Carnal and Black (1979). The buffer was 100 mM Hepes-NaOH, pH 8.0, containing 5 mM $MgCl_2$, 1 mM EDTA, 150 mM potassium acetate, 1.5% (w/v) PVP-40, and 10 mM DTT. Extracts were centifuged for 10 min at 22,000 rpm and 4°C, after which the supernatant fluid was decanted and immediately used for enzymatic assays. Because we were interested in measuring total enzymic capacity, preparations were not desalted or otherwise purified. Experience has shown that desalting results in loss of enzymic activity in these preparations and does not improve the assay. For some PEPc studies of *P. camptotricha,* leaves were homogenized on ice in cold 100 mM Hepes-NaOH (pH 7.8) buffer, containing 10 mM $MgCl_2$, 10 mM DTT, and 1% PVP-360 (w/v) to extract protein. The extracts were centrifuged for 5 min at 4°C (Sipes & Ting, 1985).

ENZYME ASSAYS. PFP and PFK assays were conducted in a 1 ml cuvette at room temperature, and PEPc was assayed in a 3 ml cuvette at 25°C by coupling with either endogenous or added malate dehydrogenase activity and following the absorbance change of NADH at 340 nm. Activity is expressed on an area basis to be consistent with the gas exchange and acid titration data. Factors are given in Table 1 to convert these data to fresh weight, chlorophyll, or a protein basis. The specific procedure for each enzyme is listed below with the compound listed last used to initiate the reaction. Chlorophyll content was determined according to Arnon (1949) and protein content was determined according to Bradford (1976).

PEP carboxylase (PEPc) (EC 4.1.1.31): 50 mM Hepes-NaOH, pH 7.8, 1 mM $NaHCO_3$, 50 mM $MgCl_2$, 0.12 mM NADH, 0.1 ml extract, and 2 mM PEP (Kluge & Osmond, 1972; Sipes & Ting, 1985). In some cases, 3 IU NAD malate dehydrogenase was added to the assay mixture.

Phosphofructokinase (PFK) (EC 2.7.1.11): 100 mM Hepes-NaOH, pH 8.0, 6 mM $MgCl_2$, 0.1 mM NADH, 15 mM fructose-6-phosphate, 6 IU aldolase, 1 IU triose phosphate isomerase, 6 IU glycerate phosphate dehydrogenase, 0.1 ml extract, and 1 mM ATP (Carnal & Black, 1979, 1983).

Pyrophosphate: fructose-6-phosphate phosphotransferase (PFP) (EC 2.7.1.90): 100 mM Hepes-NaOH, pH 8.0, 6 mM $MgCl_2$, 0.1 mM NADH, 15 mM fructose-6-phosphate, 6 IU aldolase, 1 IU triose phosphate isomerase, 6 IU glycerate phosphate dehydrogenase, 0.1 ml extract, and 1 mM PP_i (Carnal & Black, 1979, 1983).

LEAF CROSS-SECTION ANALYSIS. Fully mature fresh leaves were hand-sectioned with a blade and observed under a compound microscope. Drawings were prepared 2× actual-size using a Camera Lucida.

STATISTICAL CALCULATIONS. Statistical analyses of the survey data were performed using the ANOVA procedure in the SAS statistical package (SAS Institute, Cary, NC). The data were purged of aberrant values prior to analysis using the Q-test (Dixon, 1951). All other statistical analyses were performed using Lotus 1-2-3 (Lotus Development Corporation, Cambridge, MA) or graphed directly using Graph-Pad (Academic Press Inc., Orlando, FL).

RESULTS

SURVEY OF PEPEROMIA SPECIES FOR PHOTOSYNTHETIC MODE. Forty-five species were surveyed in this study. Based on gas exchange, diurnal acidity fluctuation, and enzymic activity, 20 species showed C_3-photosynthesis, 22 species showed CAM-cycling, and three species showed full CAM activity (TABLE 1).

In the C_3 group, gas exchange parameters, i.e., CO_2 uptake and stomatal conductance (an index of stomatal opening) were only measurable during the day. There was no significant acid fluctuation measurable in this group (TABLE 1). The C_3 group exhibited a mean PFP activity of 54.5 ± 12.3 nmol min^{-1} cm^{-2} and a mean PFK activity of 73 ± 17 nmol min^{-1} cm^{-2} (TABLE 1). The PFP/PFK ratio was 0.87 ± 0.1. PEPc activity in the C_3 group was 51.4 ± 13.2 nmol min^{-1} cm^{-2} comparable to other C_3 species (TABLE 1).

In the CAM-cycling group, gas exchange was comparable to those of the C_3 group in that all measurable CO_2 uptake was during the day. However, in contrast to the C_3 group, the CAM-cycling plants show organic acid fluctuation typical of CAM plants (TABLE 1). The CAM-cycling group showed a mean PFP activity of 134.6 ± 29 nmol min^{-1} cm^{-2} and a mean PFK activity of 121.6 ± 33 nmol min^{-1} cm^{-2}. The PFP/PFK ratio was 1.5 ± 0.3. PEPc activity in the CAM-cycling group was 119.9 ± 33.1 nmol min^{-1} cm^{-2} (TABLE 1). These enzyme assay data for the CAM-cycling group are comparable to those of the CAM group.

The CAM group were identified by typical nocturnal CO_2 uptake and nocturnal stomatal conductance with little to no measurable daytime

TABLE 1. Titratable acidity, PEPcase, PFP, and PFK activities and PFP/PFK ratios in *Peperomia* species exhibiting various photosynthetic modes.

Species or cultivar	Pathway	Acid	PFP/PFK	PFP	PFK	PEPc	Conversion factors[a] Protein	Chlorophyll	Fresh weight
		μeq g^{-1} FW		nmol min^{-1} cm^{-2}			(mg)	(mg)	(g)
"Lemon moths"	C_3	0	0.70	9.42	13.56	7.83	1.95	0.26	0.06
"Peru"	C_3	1.08	1.16	28.04	22.55	118.00	2.74	0.66	0.10
"Rio"	C_3	0	1.21	15.15	12.56	7.29	2.47	0.35	0.05
"Tam 84-2"	C_3	6.69	0.92	29.89	31.72	96.89	3.73	0.30	0.09
bicolor	C_3	0	0.98	32.74	32.62	29.99	2.13	0.44	0.09
cubensis	C_3	1.35	0.85	196.98	230.60	17.76	2.89	0.68	0.11
glabella	C_3	0	0.28	52.80	178.38	5.74	1.80	0.79	0.08
griseo-argentea	C_3	1.17	2.01	13.70	6.77	7.38	2.60	0.59	0.06
inequalifolia	C_3	0	0.69	51.43	35.75	—	7.05	0.46	0.17
maculosa	C_3	8.03	0.86	—	—	40.11	0.00	0.00	0.00
magnoliaefolia	C_3	0	0.47	21.16	45.90	44.38	4.02	0.60	0.13
martiana	C_3	0	0.67	20.03	30.46	13.74	3.09	0.66	0.13
panamensis	C_3	0	0.70	24.95	35.17	50.8	2.10	0.41	0.07
pulchella	C_3	2.96	0.58	62.22	107.00	187.15	2.98	1.02	0.13
rotundifolia	C_3	3.02	0.69	17.01	23.74	86.00	2.33	0.38	0.13
serpens	C_3	0	1.02	86.71	85.64	15.21	2.89	1.33	0.16
trinervis	C_3	4.05	0.62	11.66	18.48	28.57	3.47	0.71	0.16
trinervula	C_3	0	0.81	199.22	246.32	8.09	6.00	1.03	0.14
urocarpa	C_3	1.64	0.45	85.53	186.43	8.77	2.91	0.72	0.07
verticillata	C_3	1.97	1.74	76.42	43.9	203.56	4.94	0.62	0.23
Mean		1.60	0.87	54.48	73.03	51.43	3.10	0.60	0.11
SEM		0.51	0.09	12.27	16.95	13.16	0.33	0.07	0.01
ferreyrae	CAM	18.20	0.63	36.43	57.58	247.66	5.86	0.72	0.27
nivalis	CAM	11.18	3.39	19.58	19.58	268.24	3.49	1.24	0.43
scandens	CAM	42.12	1.09	318.99	299.85	117.11	3.01	0.72	0.33
Mean		23.83	1.70	143.22	125.67	211.00	4.12	0.89	0.34
SEM		7.65	0.70	72.31	71.67	38.64	0.72	0.14	0.04
"fallen angel"	CAM-cycling	32.60	0.75	24.85	33.21	75.26	4.31	0.36	0.06
"sp. 100"	CAM-cycling	46.34	1.81	193.02	106.74	474.56	6.64	1.12	0.43
"sp. 200"	CAM-cycling	101.07	3.28	103.19	31.45	98.60	5.31	0.55	0.14
"sp. 300"	CAM-cycling	39.87	0.71	94.91	133.19	175.62	5.38	0.63	0.25
alata	CAM-cycling	42.94	—	286.95	20.44	77.89	2.72	1.19	0.13
asperula	CAM-cycling	10.28	0.78	367.97	478.62	181.22	10.58	1.82	0.56
camptotricha	CAM-cycling	43.07	2.26	152.96	67.80	110.47	7.96	1.94	0.22
clusiifolia	CAM-cycling	4.50	1.17	22.13	18.98	8.57	6.96	2.74	0.21
congesta	CAM-cycling	21.78	1.73	102.52	57.95	—	4.03	1.51	0.33
costaricensis	CAM-cycling	5.21	0.33	9.15	27.66	159.54	3.83	0.58	0.14
crassicaulis	CAM-cycling	65.22	2.02	28.57	14.24	13.69	2.67	0.47	0.06
crassifolia	CAM-cycling	4.07	0.64	179.35	286.72	12.59	2.97	0.42	0.13
cubea	CAM-cycling	7.80	0.88	32.03	36.45	35.67	3.30	0.93	0.07
disticha	CAM-cycling	68.36	5.06	78.39	15.08	25.08	2.36	0.44	0.07
dolabriformis	CAM-cycling	29.40	0.69	191.58	277.14	630.72	8.69	1.49	0.32
fraseri	CAM-cycling	12.60	0.73	51.38	71.11	40.92	2.65	1.00	0.10
galioides	CAM-cycling	37.88	0.70	48.65	70.18	98.05	4.43	0.79	0.23
graveolens	CAM-cycling	15.70	0.96	518.38	539.35	216.57	22.00	2.32	0.63
incana	CAM-cycling	10.02	4.88	84.63	17.36	52.07	0.61	0.72	0.22
obtusifolia	CAM-cycling	9.01	1.10	369.74	337.20	17.25	3.53	0.86	0.12
pellucida	CAM-cycling	16.83	0.35	8.70	25.23	5.90	2.28	0.80	0.09
peltifolia	CAM-cycling	16.27	1.14	11.04	9.67	8.12	1.86	0.89	0.09
Mean		29.16	1.52	134.55	121.63	119.93	5.23	1.07	0.21
SEM		5.22	0.28	28.97	32.80	33.14	0.93	0.13	0.03

[a] Factors to multiply enzyme activity by to convert data to protein, chlorophyll, or fresh weight basis.

TABLE 2. One-way ANOVA results between C_3 *Peperomia* species and CAM-cycling *Peperomia* species. PFP, PFK, and PEPcase data are expressed as nmol min^{-1} cm^{-2}. Acid fluctuation is expressed as μeq g^{-1} FW.

	C_3	CAM-cycling	CAM	$P < F$
PFP	54.48 ± 12.27	134.55 ± 28.97	143.22 ± 72.31	0.037
PFK	73.03 ± 16.95	121.63 ± 32.80	125.67 ± 71.67	0.229
PFP/PFK	0.087 ± 0.09	1.52 ± 0.70	1.70 ± 0.07	0.077
PEPc	51.43 ± 13.16	119.93 ± 33.14	211.00 ± 38.64	0.086
Acid	1.60 ± 0.51	29.16 ± 5.22	23.83 ± 7.65	0.001

gas exchange. The CAM group had a mean PFP activity of 143.2 ± 72.3 nmol min^{-1} cm^{-2} and a mean PFK activity of 125.7 ± 72.7 nmol min^{-1} cm^{-2} (TABLE 1). The PFP/PFK ratio was 1.7 ± 0.7. PEPc activity in the CAM group was 211.0 ± 38.6 nmol min^{-1} cm^{-2} (TABLE 1).

One-way ANOVA analyses of enzymic data and acid fluctuation were conducted. Because of the limited number of full CAM-species identified (3 out of 45 species), the CAM species data were pooled with the CAM-cycling species data. We justify pooling of the data because both CAM and CAM-cycling showed high and similar levels of diurnal acid fluctuation and comparable levels of enzymic activity related to CAM. When pooling was done, the analysis indicated significant differences between the C_3 species and the CAM and CAM-cycling species with respect to acid fluctuation, PFP, PFK, and PEPc enzymic activity (TABLE 2).

TABLE 3 reports the identity of the photosynthetic mode of previously studied *Peperomia* species. Based on the data of TABLES 1 and 3, there are: 45 C_3 taxa, 30 CAM-cycling taxa, and 18 taxa with full CAM. Thus, of the 93 taxa studied, 52% showed aspects of CAM.

ANATOMICAL CONSIDERATIONS. Previous work by Gibeaut and Thomson (1989a, 1989b) correlated leaf anatomical features of three *Peperomia* species with previously published gas exchange and acid fluctuation data for the same three species, *P. scandens, P. camptotricha,* and *P. obtusifolia* (Hanscom & Ting, 1978; Ting & Sipes, 1985; Sipes & Ting, 1985). *P. scandens* has the most CAM-like spongy parenchyma, *P. obtusifolia* the least, and *P. camptotricha* was intermediate (Gibeaut & Thomson, 1989a, 1989b). Of the three species, *P. scandens* shows the most CAM activity and *P. obtusifolia* the least. The amount of spongy parenchyma where most of the CAM activity takes place (Nishio & Ting, 1987) is in proportion to the extent of CAM. FIGURE 1 shows some representative free-hand cross-section drawings of leaves from seven *Peperomia* species with known photosynthesis properties. Although the data are insufficient for a quantitative analysis, there appears to be some correlation between leaf anatomy and photosynthesis. The thinner-leafed species have proportionally less spongy parenchyma, e.g., *P. orba, P. argyreia,* and *P. panamensis,* and tend to be the least CAM-like. The thicker-leafed forms, e.g., *P. scandens* and *P. rauhii* = *P. congesta,* with abundant lower spongy parenchyma, are the most CAM-like. The typical window shaped leaves of *P. ferryrae, P. dolabriformis,* and *P. dolabriformis* var. *confertifolia* tend to be associated with CAM.

ONTOGENETIC SHIFT FROM C_3 TO CAM. During development of those species with CAM, the leaves shift from C_3-photosynthesis characterized by stomata that open during day to a stage in which stomata open only at night. In the initial stages, when C_3-photosynthesis is present, there is no diurnal organic acid fluctuation and the activity of PEPc is low or absent (Sipes & Ting, 1985; Holthe *et al.,* 1987). As the leaves develop, CAM activity appears as is shown for *P. camptotricha* in FIGURE 2. In the very youngest leaves, there is no appreciable acid fluctuation (FIGURE 2a) and little activity of PEPc (FIGURE 2b). As the leaves mature (older false whorls), PEPc activity increases which is accompanied by diurnal acid fluctuation. In many species, development does not progress beyond the CAM-cycling stage. In these CAM-cycling plants, the typical organic acid fluctuation of CAM and high enzymic activities of the CAM enzymes occur, but there is no nocturnal CO_2 uptake (Holthe *et al.,* 1987). Here, respiration serves as the CO_2 source for acid synthesis (Patel & Ting, 1987). In those plants that develop full CAM activity, subsequent to the synthesis of the CAM enzymes and the commencement of diurnal organic acid fluctuation, a shift to nocturnal stomatal opening and nocturnal gas exchange typical of full CAM occurs (Sipes & Ting, 1985; Holthe *et al.,* 1987).

If plants are water-stressed during leaf development, there appears to be an acceleration of the CAM induction since PEPc activity appears in younger leaves at a higher level than nonwater-stressed control plants (FIGURE 2b). This observation is similar to that of the salt and water-stress induction of CAM in *Mesembryanthemum* (Winter, 1973). However, because stomata rap-

TABLE 3. Summation of published research on the photosynthetic modes of *Peperomia* species.

Species	Mode	Source
angulata (=*quadrangularis*)	C_3	A
argyreia E. Morr.	C_3	A
arifolia Miq.	C_3	A
bernieriana Miq.	C_3	A
bicolor Sodiro.	C_3	A, K
boivinii C. DC.	CAM	A
camptotricha Miq.	CAM-cycling	G, K
caperata Yunck.	C_3	A
caulibarbis Miq. (=*jimenesana* (C. DC.) Trel.)	CAM-cycling	A
clusiifolia (Jacq.) Hook.	C_3	A, K
columella Rauh & Hutch.	CAM	A
congesta HBK (=*rauhii*)	CAM	A, K
crassicaulis F.&R.	CAM-cycling	I, K
crassicaulis F.&R.	C_3	A
crassifolia Bak.	C_3	A
cuspidilimba C. DC.	C_3	A
dolabriformis HBK	CAM	A
ferreyrae Yunck.	CAM	I, K
flexicaulis var. *microphylla* Wawra.	CAM	A
fraseri C. DC.	CAM-cycling	B, E, K
galioides HBK	C_3	A
glabella (Swartz) A. Dietr.	CAM-cycling	A, I
graveolens Rauh & Barth.	CAM	A
griseo-argentea Yunck.	C_3	A, K
hoffmannii C. DC.	C_3	A
incana (Haw.) Hook.	CAM-cycling	A, K
johnsonii C. DC.	C_3	D
leptostachya Hook. & Arn.	C_3	A, D
longespicata C. DC.	CAM	A
macrostachys (Vahl.) A. Dietr.	CAM-cycling	G
maculosa (L.) Hook.	C_3	A, K
magnoliaefolia (Jacq.) A. Dietr.	CAM-cycling	A, I
magnoliaefolia var. *tithymaloides*	CAM-cycling	A
marmorata Hook.	C_3	A
metallica L. Linden & Rodig.	C_3	A
microphyllaphora Trel. & Yunck.	CAM	I
nivalis Miq.	CAM	A, H, K
obtusifolia (L.) A. Dietr.	CAM-cycling	A, C, E, K
oerstedii C. DC.	CAM-cycling	G
orba Bunt.	C_3	B, E
panamensis C. DC.	C_3	A, K
panamensis C. DC.	CAM-cycling	G
pellucida (L.) Kunth.	CAM	I
pellucida (L.) Kunth.	C_3	J
pellucida (L.) Kunth.	CAM-cycling	A, K
peltifolia C. DC.	CAM-cycling	E, K
pereskiaefolia (Jacq.) HBK	CAM	A
polybotrya HBK	C_3	A
polystachyoides Dablst.	C_3	A
ppucu-ppucu Trel.	C_3	A
puberulispica C. DC.	CAM	A
quadrangularis (J. V. Thomps.) A. Dietr.	C_3	A
resedaeflora Lind. & André	C_3	A
rotundifolia (L.) HBK	CAM-cycling	G
rotundifolia (L.) HBK	CAM	A
rubella (Haw.) Hook.	C_3	A
scandens Ruiz & Pavón	CAM	B, E, F, K
serpens (Swartz) Loud.	CAM	A
serpens (Swartz) Loud.	CAM-cycling	G
sp.	CAM	H
sp. nov.	CAM-cycling	G

TABLE 3. Continued.

Species	Mode	Source
tetraphylla (Forst. f.) Hook. & Arn.	C_3	D
trinervis Ruiz & Pavón	C_3	A, K
verschaffeltii Lem.	C_3	A
verticillata (L.) A. Dietr.	CAM	A

[a] Sources: A, Starnecker (1984) Methods 1, 5. B, Ting *et al.* (1987), Methods 1, 2, 3, 5, 6. C, Hanscom and Ting (1978), Methods 1, 2. D, Winter *et al.* (1983), Method 3. E, Sternberg *et al.* (1984), Methods 3, 4. F, Holthe *et al.* (1987), Methods 1, 2, 3. G, Ting *et al.* (1985), Methods 1, 2, 3, 4. H, Virzo de Santo *et al.* (1983), Method 5. I, Ting and Hann (unpubl.), Methods 6, 7. J, Ehleringer *et al.* (1987), Method 3. K, Holthe (1988; this paper), Methods 1, 2, 6, 8. Methods: 1, gas exchange. 2, titratable acidity. 3, ^{13}C. 4, ^{2}H. 5, malic acid. 6, PEPc. 7, anatomical. 8, PFP/PFK.

idly close in response to drought (Ting, 1985) which prevents uptake of CO_2, the fluctuation of organic acids is much reduced (FIGURE 2a).

SEASONAL ASPECTS OF CAM IN PEPEROMIA. Monthly titration of extractable organic acids indicated that plants grown in the glasshouse in Riverside showed a definite seasonal pattern of CAM activity. Both *P. camptotricha* and *P. scandens* showed maximal diurnal fluctuation of organic acids in the spring and summer (FIGURE 3). During the year, large organic acid fluctuations are generally associated with high activities of PEPc (data not shown). We cannot be certain from these data whether or not there would be a seasonal aspect to CAM in *Peperomia* growing in natural tropical environments which would tend to have little variation in temperature and light throughout the year in comparison with Riverside.

DISCUSSION

Peperomia is a large pantropical genus with over 600 species (Lawrence, 1955), of which 70% or more are epiphytic (Madison, 1977). Most of the nonepiphytic species occur naturally on rock outcrops or shallow soils. Of the 93 taxa reported to date, 45 were judged to show C_3-photosynthesis, 30 to show CAM-cycling, and 18 to show full CAM. Thus, over 50% of the taxa investigated show some aspects of CAM. Although this sample represents a small percentage of the total

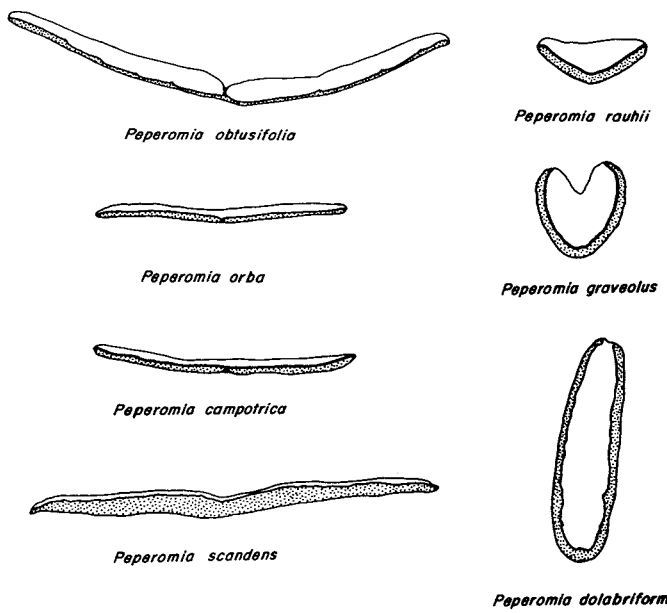

FIGURE 1. Representative Camera Lucida drawings of cross-sections from various C_3, CAM-cycling, and CAM *Peperomia* species. Refer to TABLES 1 and 3 for photosynthetic mode.

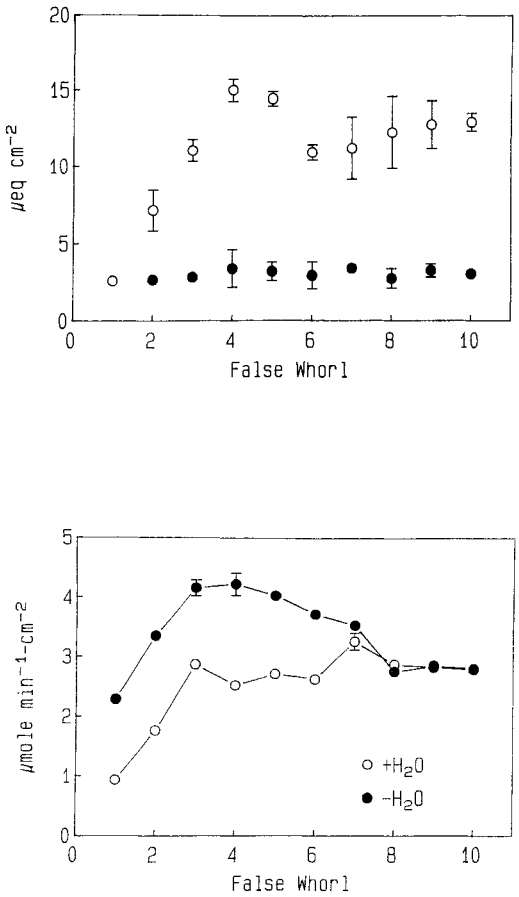

FIGURE 2. Diurnal organic acid fluctuation and PEPc activity in developing leaves of control and water-stressed *P. camptotricha* (whorl 1 is the youngest). 2a. Diurnal acid fluctuation. 2b. Activity of PEPc. Open circles = well-watered controls; closed circles = water-stressed. Error bars are ±SEM.

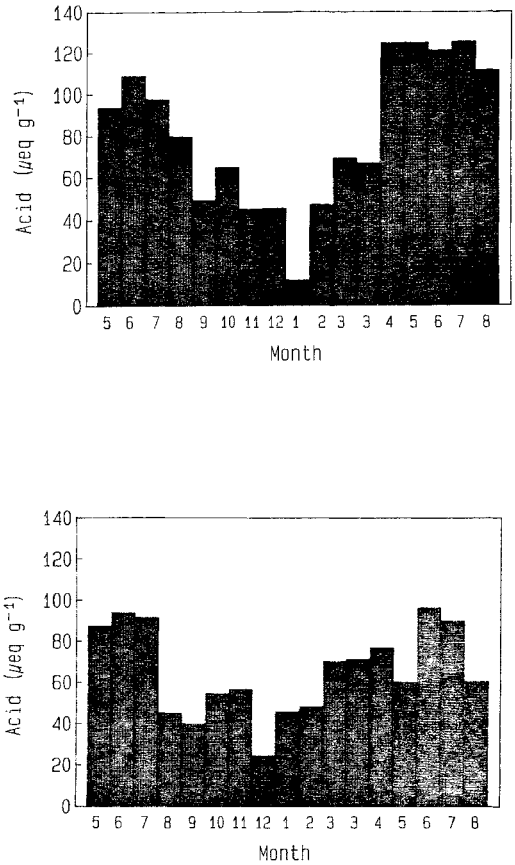

FIGURE 3. Diurnal organic acid fluctuation of *P. camptotricha* (upper panel) and *P. scandens* (lower panel) on a monthly basis for two spring and summer seasons and one winter (1985–1986).

known species, the survey is probably representative enough to conclude that CAM is an important aspect of the ecophysiology and biochemistry of the genus *Peperomia.*

CAM has been documented in many epiphytic flowering plants (Coutinho, 1963; Guralnick *et al.,* 1986; McWilliams, 1970; Medina, 1974; Sinclair, 1984; Winter *et al.,* 1983). CAM occurs in the epiphytic species of the Orchidaceae, Bromeliaceae, Cactaceae, and the Gesneriaceae. In our recent study of photosynthesis of the epiphytic flowering plants on the tropical island of St. John, over 75% showed CAM (Ting, 1989). Thus, it is not surprising that *Peperomia* also have a large number of species with CAM and/or CAM-cycling.

CAM is generally assumed to be a physiological adaptation to drought in terrestrial plants (Kluge & Ting, 1978; Ting, 1985). The important physiological consequence of CAM is that the stomata are open at night and closed during the day when evaporative demand is highest. Since most gas exchange, CO_2 uptake, and water loss occur at night, CAM plants lose much less water than C_3 plants, frequently as much as 100 times less (Kluge & Ting, 1978; Osmond, 1978; Ting, 1985). CAM in *Peperomia,* as in other epiphytes, is an adaptation to water-stressed environments (Sinclair, 1984; Ting, 1989).

In an ecological context, the phenomenon of CAM-cycling is more difficult to interpret than CAM because gas exchange takes place during the day when water loss is excessive (Ting & Sipes, 1985). In *P. scandens,* CAM-cycling is a developmental stage of young leaves intermediate between C_3-photosynthesis and CAM

(Holthe *et al.*, 1987). As leaves of *P. scandens* mature, the progression from C_3 to CAM is: C_3 → CAM-cycling → CAM. Those plants which do not develop beyond CAM-cycling may be incipient CAM plants in an evolutionary sense. We previously suggested that CAM-cycling may be a metabolic state poised to shift from CAM-cycling to CAM or CAM-idling when the plants are water-stressed (Ting & Sipes, 1985). In this context, Martin *et al.* (1988) proposed that CAM-cycling results in carbon conservation, and as a consequence, water conservation.

Many succulent species shift from C_3 to CAM in response to photoperiod, water-stress, salt-stress, high temperature-stress, and during development (Ting, 1985). Because *Peperomia* species also respond to photoperiod (Sipes & Ting, 1985) and water-stress (Hanscom & Ting, 1978; Sipes & Ting, 1985), and may shift photosynthetic mode during development (Holthe *et al.*, 1987), it is somewhat difficult to assign a particular species to C_3, CAM-cycling, or to CAM without extensive studies involving a variety of environmental conditions and plant ages. These shifts in photosynthetic mode in response to environmental perturbations and/or development could account for the different assignments between previous studies and those reported here. For example, *P. orba* was classified as C_3 by Ting and Sipes (1985) and is used as the standard for a C_3 *Peperomia* in our laboratory. However, hydrogen isotopic composition data led us previously to predict that it was CAM-cycling (Sternberg *et al.*, 1984). *Peperomia rotundifolia* was determined to be CAM-cycling by Ting *et al.* (1985) and CAM by Starnecker (1984). Between the work presented here and that of Starnecker (1984), 22 species were surveyed in duplicate. Twelve species were in agreement. Of the remaining ten species, seven were shown here to be more C_3-like than reported by Starnecker and three were shown to be more CAM-like. Although these discrepancies could be due to experimental error, it is more likely that the differences are the result of growing conditions or interpretation of the data since Starnecker did not report organic acid fluctuation data for all species. There is also the possibility that the various taxa studied were not comparable. Nevertheless, these studies illustrate the ambiguity in assigning a specific photosynthetic pathway to a species when it is known that both the developmental stage and environmental perturbations influence the metabolism. Another complication of ascertaining the photosynthetic mode of *Peperomia* is illustrated by the seasonal variation of CAM reported here with *P. scandens* and *P. camptotricha* growing in glasshouses in Riverside. Even if this seasonality is an artifact of our greenhouse growing conditions, it would influence the measurements taken at various times during the year. At this time, however, it seems reasonable to conclude that over 50% of *Peperomia* species are CAM or CAM-cycling. Whether all species have the capacity for CAM under appropriate environmental or seasonal conditions cannot be stated for certain.

It is possible that all species of *Peperomia* have some capacity for CAM. Previously, we reported the hydrogen and carbon isotope composition of 13 species of *Peperomia* and two morphological variants of two of the species (Ting *et al.*, 1985). The data indicated that the carbon isotope composition of the *Peperomia* species was within the range reported for C_3-species ($-26‰$ for the *Peperomia* spp. and $-28.2‰$ for ten C_3-species), but the hydrogen isotope composition was positive similarly to CAM plants ($-80‰$ for C_3-species and $-25‰$ for C_4-species as opposed to $+2‰$ for the *Peperomia* spp. and $+30‰$ for 21 CAM species). The C_3-like carbon isotope composition for *Peperomia* was interpreted to be the result of large quantities of exogenous CO_2 assimilated during the day. However, the positive hydrogen isotope composition was indicative of CAM. The carbon isotope composition is the result of fractionation during diffusion of CO_2 and by discrimination during carboxylation (O'Leary, 1981). Hydrogen isotope fractionation is related to carbohydrate metabolism of CAM species (Sternberg *et al.*, 1984) and possibly related to the relatively high levels of PFP reported here for *Peperomia* (Carnal & Black, 1979, 1983).

Additional studies could be directed toward determining if *Peperomia* species with similar photosynthetic modes are related phylogenetically. Also, it would be interesting to ascertain if they occur in similar geographic regions or if there is an environmental or ecological basis for the particular photosynthetic mode expressed by a species. Since CAM is an important physiological adaptation to water-stress (Kluge & Ting, 1978) and many epiphytes experience frequent and periodic drought (Sinclair, 1983, 1984), a causal link to environment is a reasonable hypothesis that could be tested with further research.

Acknowledgments

The research reported here was supported by the U.S. National Science Foundation (DCB-88007860). The survey research is from P. A. Holthe. 1988. Photosynthetic variation in the Peperomiaceae. Ph.D. dissertation, University of California, Riverside. We thank Ms. Jan Lip-

pert for the Camera Lucida drawings of *Peperomia* leaves. We also thank Ms. Janet Hann Giles for laboratory assistance.

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SYSTEMATICS AND EVOLUTION OF THE TROPICAL-SUBTROPICAL TILLANDSIA SUBGENUS DIAPHORANTHEMA (BROMELIACEAE)

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ABSTRACT. All currently known taxa of *Tillandsia* subgenus *Diaphoranthema* have been studied and treated in a taxonomic revision and other papers. Six groups of species ("aggregates"), distinguished by floral morphology and certain vegetative features, are recognized. These groups contain a series of taxa that display morphological reduction accompanied by shortening of life cycles (neoteny). Polyploidy is frequent within *Diaphoranthema.* Compared with subgenus *Phytarrhiza,* the closest related subunit of the genus *Tillandsia,* neotenic species of *Diaphoranthema* have migrated into more isolated and climatically extreme areas than have any other *Tillandsia* species. Abbreviated life cycles and strong tendencies of autogamy and cleistogamy, in connection with increasing polyploidy, are proposed to be responsible for the high stress tolerance and dispersibility of the species of *Diaphoranthema.*

Sistemática y evolución en *Tillandsia* subgenero *Diaphoranthema* (Bromeliaceae).

RESUMEN. Todos los taxa que actualmente son conocidos fueron estudiados y fueron tratados en una revisión taxonómica y otras publicaciones. Seis grupos de especies ("aggregates"), que demuestran neotenia progresiva son acceptados según sus morfologías florales y vegetativas. Poliploidía existe con frecuencia en el subgénero *Diaphoranthema.* Comparado con el subgénero *Phytarrhiza,* que es el pariente próximo, las especies neotenas de *Diaphoranthema* se han propagado hacia áreas mas aisladas y hacia zonas climáticas mas extremas que otras especies del genero *Tillandsia.* Ciclos biológicos abreviados y tendencias pronunciadas de autogamia y de cleistogamia son propuestos como responsables de la tolerancia pronunciada contra "stress" y de la propagación de las especies de *Diaphoranthema.*

INTRODUCTION

The large neotropical genus *Tillandsia* L., which currently comprises about 550 species, is distributed from southern United States to Central Argentina and Chile. It has been divided several times into subunits at various taxonomic levels and even into separate genera (Smith & Downs, 1977). In the most modern monographic treatment (Smith & Downs, 1977), seven subgenera are accepted. They only represent natural alliances in part, as this work was based mainly on herbarium studies. Recent investigations (Till, 1984; Gilmartin & Brown, 1986a) have revealed that the circumscriptions of the subgenera should be revised in some cases. Classifications will be altered in the light of discoveries from research on flower morphology, pollen and stigma structures, cytology, isozymes, and cp-DNA. Very little is known about pollination types, breeding systems, and ecology.

MATERIALS AND METHODS

This paper deals with the highly specialized subgenus *Diaphoranthema* (Beer) Baker, a xeric group with Bolivian-Argentinian centers of distribution. The following research has been done (Till, 1984, 1989a, 1989b, 1991): thorough morphological analysis of inflorescences, flowers, and vegetative traits of living plants; study of anthesis in cultivation and in the field; germination tests; and extensive herbarium studies, including examination of all types. The herbarium vouchers and the type specimens allowed the interpretation of the taxa accepted within this subgenus and provided distribution maps. After a taxonomic revision of *Diaphoranthema* (Till, 1984, 1989a, 1989b, 1991), six species groups have been recognized, and several new taxa have been described (Till & Hromadnik, 1983, 1984; Hromadnik & Till, 1991; Till, 1992). Caryological examination (Till, 1984) revealed a high degree of polyploidy upon the currently accepted base number of $x = 25$ (Marchant, 1967; Till, 1984; Brown & Gilmartin, 1989a). The distributions of individual species have been compared with the species group concept and the ploidy levels, but only a selected example is represented here.

The distributions of the species of *Diaphoranthema* cannot be interpreted correctly without also considering the xeric members of subgenus *Phytarrhiza* (Visiani) Baker (e.g., *T. streptocarpa* Baker, *T. kurt-horstii* Rauh, *T. reichenbachii* Baker, *T. duratii* Visiani, *T. arhiza* Mez in C. DC., *T. paleacea* Presl, *T. marconae* W. Till & E. Vitek, *T. kirschnekii* Rauh & W. Till, *T. cacticola* L. B. Smith, *T. purpurea* Ruiz Lopez & Pavón y Jiménez, *T. humilis* Presl, *T. aurea* Mez, *T.*