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Induction by drought of crassulacean acid metabolism in the terrestrial bromeliad, *Puya floccosa*

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Abstract

In the terrestrial bromeliad, *Puya floccosa*, a value of carbon isotopic composition ($\delta^{13}C$) of –22‰ has been previously reported, suggesting the operation of weak and/or intermediate (C_3-CAM) crassulacean acid metabolism (CAM). In order to characterize the operation of CAM in *P. floccosa* and its possible induction by drought, plants were grown in Caracas and subjected to four independent drought cycles. Additionally, since plants of this species grow in Venezuela in a large range of elevations, leaf samples were collected at elevations ranging from 725 to 2,100 m a.s.l. in the Venezuelan Andes and the Coastal Range, in order to evaluate the effect of elevation on CAM performance. Even though nocturnal acid accumulation occurred in both watered and droughted plants, mean ΔH^+ was higher in droughted than watered plants [ΔH^+ = 60.17.5 and 22.9 ± 5.2 μmol g^{-1}(FM), respectively]. The majority of plants from all the natural populations sampled had low values of $\delta^{13}C$ not differing significantly from those of C_3 plants collected as standards and $\delta^{13}C$ did not change with elevation. We conclude that *P. floccosa* is capable of a weak CAM activity, with a large variability among populations and drought experiments probably due to local and temporal differences in microclimatic variables and drought stress; elevation bears no influence on values of $\delta^{13}C$ in this species.

Additional key words: *Bromeliaceae,* elevation, nocturnal acid accumulation.

Introduction

Crassulacean acid metabolism (CAM) is widely viewed as an adaptation to water-deficit-prone environments. Four variants are currently recognized: (*1*) obligate CAM, with high nocturnal acid accumulation (ΔH^+) and CO_2 fixation; (*2*) facultative or inducible CAM, also known as C_3-CAM, with a C_3 form of CO_2 fixation and nil ΔH^+ in the noninduced state, and nocturnal CO_2 fixation and ΔH^+ in the induced state; (*3*) CAM-cycling, with daytime CO_2 fixation and ΔH^+ but no nocturnal stomatal aperture, and (*4*) idling, with small ΔH^+ and stomatal closure during the entire day and night in severely stressed plants (Herrera 2009). Strong and weak CAM can be differentiated on the basis of the magnitude of ΔH^+ (Herrera 2009), an exception being the C_3-CAM plant *Clusia minor*, in which values of ΔH^+ as high as 1.4 M have been measured (Borland *et al*. 1992).

CAM is widespread among the Bromeliaceae (Martin 1994, Benzing 2000). In the Pitcairnioideae, a wide range of $\delta^{13}C$ values indicate that the obligate CAM, C_3 and C_3-CAM modes of carbon fixation are present. Values of $\delta^{13}C$ of 18 species in the genus *Puya* (Bromeliaceae, Pitcairnioideae) examined in South America suggest wide variations from CAM to C_3 carbon fixation pathways, with a mean of –21.5 ± 0.8‰ (Martin 1994), which lies between full CAM plants (–12.4‰ in the Pitcairnioideae *Dyckia tuberosa* and *Encholirium hoehneanum*; Medina *et al.* 1977) and purely C_3 plants (–30‰ in species of *Pitcairnia*; Medina *et al.* 1977), thus suggesting C_3-CAM intermediacy. A review of 24 inducible CAM species (Herrera 2009) shows that values of $\delta^{13}C$ are around –23.9 ± 0.9‰, with a maximum of –14 and a minimum of –30‰.

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Abbreviations: CAM – crassulacean acid metabolism; Chl – chlorophyll; DM – dry mass; FM – fresh mass; FM/A – fresh mass per area; RH – relative humidity; T – air temperature; S_m – mesophyll succulence index; TR – total radiation; TR_{int} – daily integrated TR.

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P. floccosa (Linden) E. Morren ex. Mez has the widest geographic and elevation range of any of the species in the genus. It grows in Costa Rica, Colombia, Venezuela, and Brazil (Benzing 2000) and in Venezuela, it can be found from 500 to 3,800 m at locations with marked differences in day/night temperature. The plants grow exclusively on bare soil under full-sun exposure. Relatively high values of $\delta^{13}C$ (–22.0‰; Medina and Troughton 1974) and nocturnal malate accumulation [18 μmol g^{-1}(DM), or approximately 120 μmol g^{-1}(FM)] but nil nocturnal CO_2 uptake have been reported (Medina 1974) for this species.

Varying degrees of CAM have been found in high-elevation species. CAM was reported to occur in plants of *Echeveria columbiana* (Crassulaceae) growing at *ca*. 4,000 m in the Venezuelan Andes (Medina and Delgado 1976). Two tropical cacti growing between 4,000 and 4,700 m had values of ΔH^+ and $\delta^{13}C$ that are suggestive of obligate CAM (Keeley and Keeley 1989). In a survey carried out on Portulacaceae of the Chilean Andes, species with $\delta^{13}C$ higher than –23‰ were found mainly at lower elevations (1,800–3,100 m) while those with $\delta^{13}C$ lower than –24‰ grew from 3,300 to 4,400 m (Arroyo *et al*. 1990). The question arises whether $\delta^{13}C$ and therefore CAM activity in *P. floccosa* change with elevation.

In order to investigate the occurrence of intermediate C_3-CAM metabolism in *P. floccosa* through determinations of nocturnal acid accumulation (ΔH^+) and the influence of drought on CAM performance, we cultivated plants under semicontrolled conditions in Caracas at 1,000 m and subjected them to drought. We also examined the effect of elevation on CAM performance through the determination of $\delta^{13}C$ of plants growing at six elevations, from 725 to 2,110 m, in the Venezuelan Andes and the Coastal Range. Nocturnal acid accumulation was also determined at three elevations in the Coastal Range during the dry and the rainy season.

Materials and methods

Experiments: Ten plants of *P. floccosa* collected at the Henri Pittier National Park (Rancho Grande) at 725 m (10º19' N–67º39' W) were planted in 10-l plastic pots containing commercial garden soil and grown under full sun exposure in the garden under a semitransparent plastic roof in the Instituto de Biología Experimental, Caracas (1,000 m a.s.l.). Air temperature (T), relative humidity (RH) and total radiation (TR) were collected by a weather station model *Vantage Pro* (*Davies Instruments*, Hayward, CA, USA). Plants were frequently watered during six months before beginning the experiments, which consisted of subjecting plants to water deficit for up to 20 days. Four nonconsecutive drought cycles were conducted on the same plants.

Measurements of physiological variables were made in the middle portion of the leaf lamina. The cross-sections of fresh leaves (n = 6, one leaf per plant) were free-hand prepared and observed under a microscope at 200 × magnification. The ΔH^+ was determined as the difference between values of H^+ content at dawn and dusk in the leaf sap obtained from frozen leaf segments of known fresh mass (FM) which were placed in plastic 10-ml syringes, centrifuged at 4,600 × g for 10 min and titrated with 1 mM KOH to pH 7 (Nobel 1988). Fresh mass per area (FM/A) and dry mass (DM) per area (after drying samples for 72 h at 60ºC) of disks of known area were measured at 07:00 h. Chlorophyll (Chl) content was determined in 80% cold acetone extracts of leaf sections collected at 18:00 h according to Bruinsma (1963). Mesophyll succulence index (S_m) was calculated after Kluge and Ting (1978) as the ratio of leaf water content to Chl content of the mesophyll.

Field studies: Leaves were collected in the Venezuelan Andes and Rancho Grande. Sites differed in elevation, rainfall, and temperature. The collection sites in the Andes comprised Táchira and Mérida states, located at 8° N–72° W. In Rancho Grande, measurements were made on the road from Maracay to Cata at an elevation of 725 m (10º19' N–67º39' W) in April 2002 (dry season) and July 2002 (rainy season), and on the road from Maracay to Choroní at elevations of 725 (10º18' N–67º33' W), 1,150 and 1,400 m in January 2005 (rainy season) and March 2005 (dry season). The dry season in Rancho Grande spans from December to April, with mean monthly rainfall of 55 mm; the mean monthly rainfall for the rainy season is 290 mm (Huber 1986). Samples of C_3 plants were collected at each site to serve as standards for $\delta^{13}C$ values.

Natural carbon isotope discrimination: Dried leaf samples were ground and analyzed by mass spectrometry for $\delta^{13}C$ at Centro de Energia Nuclear na Agricultura, Universidad de São Paulo, Laboratório de Ecología Isotópica, Piracicaba (Brasil) and Paleolab, University of Massachussets, Boston (USA).

Statistics: Unless otherwise stated, all measurements were replicated six times. Significance was assessed using the Statistica package by one- and two-way *ANOVA*s at $p<0.05$.

Results

Leaf cross-sections showed the presence of frequent chlorenchyma bundles separated by colenchyma arranged below a thick Chl-less hydrenchyma which comprised 39 ± 2% of the leaf thickness. Mesophyll succulence index was calculated based on mesophyll water content, assuming that FM of the mesophyll was 61% of whole-leaf FM, since the mesophyll amounted to that proportion of the whole-leaf thickness. The S_m ranged from 6.2 ± 1.2 in watered plants in the garden to 2.4 ± 0.2 g(H_2O) mg^{-1}(Chl) after 14 d of drought.

Garden experiments: The four garden experiments did not differ largely in values of microclimatic variables; FM/A was in two experiments 1.7 times higher than in the other two (Table 1), higher FM/A indicating a lower rate of plant dehydration. Values of ΔH^+ were corrected for the presence of the hydrenchyma, assuming that acids accumulate only in the chlorenchyma. The mean maximum ΔH^+ found in the garden experiments after 6–10 d of drought was 67.1 ± 9.4 µmol g^{-1}(FM). In all four experiments, ΔH^+ decreased after this length of time.

A high determination coefficient was found between ΔH^+ and daily integrated TR, TR_{int} (r^2 = 0.51; 71% of cases in the confidence interval at p=0.99; Fig. 1), but not maximum air temperature, minimum air temperature, minimum relative humidity or FM/A (r^2<0.13 for all four variables).

Table 1. Values in garden experiments of maximum nocturnal acid accumulation (ΔH^+), fresh mass per area (FM/A), daily integrated total radiation (TR_{int}), maximum and minimum air temperature and minimum relative humidity (RH) for four independent experiments done on the dates indicated. Values are mean ± SE (n = 6 for ΔH^+ and FM/A, and n = 4 for microclimatic variables) taken on the date and three more dates around it.

	Date 18/02/2003	21/09/2003	10/10/2005	27/10/2006
ΔH^+ [µmol g^{-1}(FM)]	57.3 ± 10.9	110.1 ± 25.3	34.5 ± 8.6	45.0 ± 6.8
FM/A [mg cm^{-2}]	23.2 ± 1.5	43.4 ± 4.7	40.3 ± 1.9	26.7 ± 1.3
TR_{int} [kW m^{-2} d^{-1}]	3.4 ± 0.8	4.3 ± 0.4	3.0 ± 0.6	3.0 ± 0.7
maximum air T [ºC]	27.4 ± 0.2	30.3 ± 0.9	28.3 ± 1.3	24.8 ± 1.6
minimum air T [ºC]	15.3 ± 1.2	20.3 ± 0.6	20.2 ± 0.7	18.9 ± 0.4
minimum RH [%]	47.7 ± 4.4	50.5 ± 2.5	51.3 ± 5.8	63.3 ± 5.0

Field studies: The average $\delta^{13}C$ in plants of *P. floccosa* from all the populations sampled was –28.3 ± 0.2‰ (Table 2), with a minimum of –31.4‰ and a maximum of –23.1‰. These values were not significantly different from those of C_3 plants collected as standards, regardless of elevation or macroclimate, with the exceptions of the 725-m-high population (higher in *P. floccosa*) and 1,150-m-high population in Rancho Grande (lower in *P. floccosa*) during the rainy season. At the Rancho Grande 725-m-high population, $\delta^{13}C$ was lower during the rainy than the dry season.

During the dry season in the Rancho Grande sites, dawn H^+ content was found to be significantly higher than dusk values at 1,400 m; the highest ΔH^+ was measured at 1,400 m (Fig. 2). During the rainy season, dawn and dusk values were not significantly different among elevations and ΔH^+ was close to zero. The ΔH^+ was significantly different between seasons and elevations only at 1,400 m.

When results from garden experiments and field determinations were plotted together (Fig. 3), a large variability with FM/A was found in H^+ content and ΔH^+; dawn and dusk values were significantly different (p<0.001). Values of $\Delta H^+ \geq 50$ µmol g^{-1}(FM) in droughted ± plants (44% of data points) occurred at FM/A = 37 ± 2 mg cm^{-2}. Even though nocturnal acid accumulation occurred in both watered and droughted plants, mean ΔH^+ was higher in droughted than in watered plants [ΔH^+ = 60.1 ± 7.5 and 22.9 ± 5.2 µmol g^{-1}(FM), respectively].

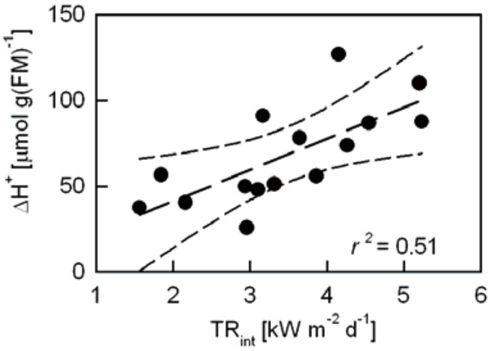

Fig. 1. Changes with daily integrated total radiation in maximum nocturnal acid accumulation of plants of *Puya floccosa* subjected to drought in the garden. Values are data points from four experiments. The regression line (*solid line*), confidence intervals *(broken lines*) and determination coefficient (p<0.05) are indicated.

Discussion

The occurrence of CAM in plants of *P. floccosa* was assessed both *in situ* and under semicontrolled conditions through the measurement of ΔH^+. Previous reports indicated that *P. floccosa* showed some degree of CAM (Medina 1974, Medina and Troughton 1974); ours is a more thorough study of CAM induction by drought and the effect of elevation on it. Relatively low values of $\delta^{13}C$ were indicative of the C_3-CAM mode, in agreement with a report whereby four species of *Puya* are placed in a C_3-CAM mode clade (Reinert *et al.* 2003). In the present investigation, the great majority of values of $\delta^{13}C$ in *P. floccosa* were not significantly different from those of C_3 standard species, indicating that the contribution of CAM to biomass production was negligible.

A large variability in values of ΔH^+ was found in plants under field or garden conditions and under either natural or imposed drought; in general, ΔH^+ was high at intermediate values of FM/A. This variability in ΔH^+ in *P. floccosa* resembles that observed in *C. minor*, in which ΔH^+ changed very dynamically in response to water supply (Herrera *et al.* 2008). In *P. floccosa*, drought promoted nocturnal acid accumulation, with droughted plants having an average value 2.6 times higher than that in watered plants.

Values of S_m were higher than 1 g(H_2O) mg^{-1}(Chl) and maximum ΔH^+ was 233 μmol g^{-1}(FM), typical of many full CAM species (Kluge an Ting 1978). These observations support the hypothesis of the occurrence of

Table 2. Values of natural carbon isotope composition of plants of *Puya floccosa* collected at locations in Venezuela differing in elevation and yearly mean air temperature (T). Values of $\delta^{13}C$ are means ± SE ($n \geq 3$). *Different letters* indicate significant differences ($p < 0.05$). Rainfall and temperature data for Rancho Grande taken from Huber (1986).

Location	Season	Species	Elevation [m]	Min/max T [°C]	Rainfall [mm (yr)$^{-1}$]	$\delta^{13}C$ [‰]
La Auyamala	dry	*P. floccosa*	2,110	18–26	1,200	-27.2 ± 0.2^{de}
		Vaccinium ssp.				-27.6 ± 0.3^{cde}
Queniquea	dry	*P. floccosa*	1,600	12–18	1,200	-28.3 ± 0.4^{cde}
		Miconia ssp.				-26.8 ± 0.5^{e}
Barinitas	dry	*P. floccosa*	500	12–18	1,500	-28.0 ± 0.3^{cde}
		Miconia ssp.				-26.8 ± 0.7^{e}
Rancho Grande	dry	*P. floccosa*	725	18–25	1,750	-27.6 ± 0.1^{cde}
		Tithonia diversifolia				-29.5 ± 0.1^{bc}
	rainy	*P. floccosa*	725	18–25		-29.4 ± 0.2^{bc}
		Tithonia diversifolia				-31.1 ± 0.3^{a}
	rainy	*P. floccosa*	1,150	15–22		-30.5 ± 02^{ab}
		Miconia ssp.				-28.8 ± 0.4^{c}
	rainy	*P. floccosa*	1,400	11–17		-28.5 ± 0.3^{cd}
		Miconia ssp.				-29.1 ± 0.7^{bc}

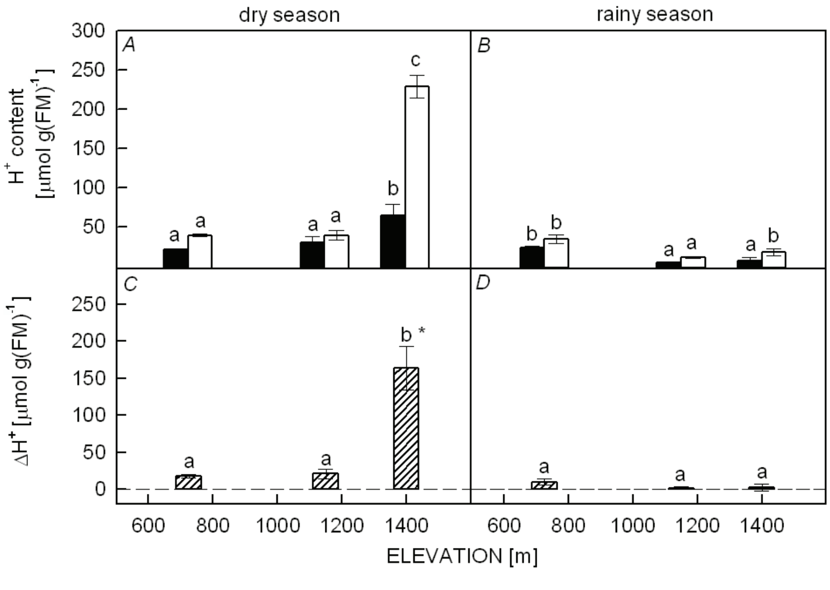

Fig. 2. Changes with elevation of populations of *Puya floccosa* growing at Rancho Grande during the dry and the rainy season in: *A,B*: dusk (*filled bars*) and dawn (*empty bars*) proton content, and *C,D*: nocturnal proton accumulation (*hatched bar*). Values are means ± SE ($n = 6$). *Different letters* indicate significant differences ($p < 0.05$) at each season; an asterisk indicates significant differences due to elevation and season.

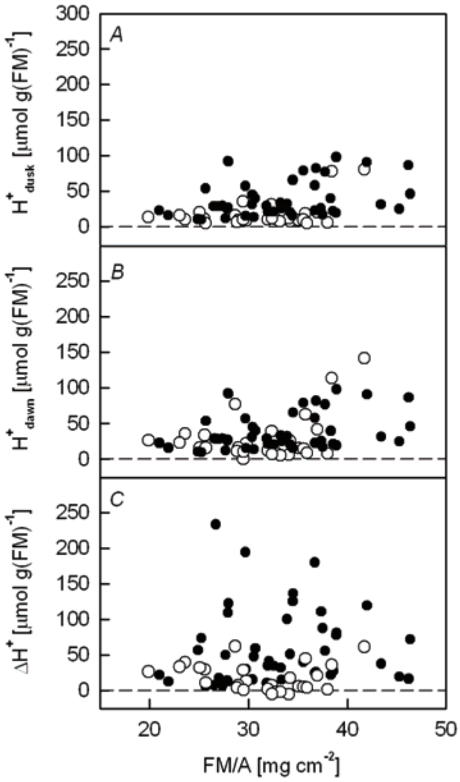

Fig. 3. Changes with fresh mass per area (FM/A) in watered and droughted plants of *Puya floccosa* measured under natural conditions and in the garden in *A*: dusk proton content; *B*: dawn proton content and *C*: nocturnal proton accumulation. Values are individual data points of four independent field studies and four independent drought experiments in the laboratory. *Open symbols* – watered plants and plants during the rainy season; *closed symbols* – droughted plants.

CAM in this species.

No effect of elevation on $\delta^{13}C$ was found in *P. floccosa*. Several Clusiaceae sampled along an elevation gradient in Venezuela had values of carbon isotope discrimination indicative of the occurrence of CAM exclusively at or below 1,500 m (Diaz *et al.* 1996). Similarly, populations of *Sedum wrightii* growing along an altitudinal range in southwestern USA along an altitudinal range had the highest $\delta^{13}C$ at 1,500 and 2,400 m and the lowest at 3,600 m (Kalisz and Teeri 1986). These findings suggest a varied response of CAM to elevation.

Slightly higher values of $\delta^{13}C$ in *P. floccosa*, not significantly different from that of its C_3 standard, during the dry relative to the rainy season in the 725-m population at Rancho Grande may reflect an increase in water-use efficiency (Farquhar and Richards 1984) rather than in CAM activity, since ΔH^+ was not significantly different between seasons.

Differences in ΔH^+ along the altitudinal transect in Rancho Grande during the dry season may have been due to differences in nocturnal minimum T, which was considerably lower at the 1,400-m-high location than at the 725- and 1,150-m-high locations. The stimulatory effect of low night-time temperature on ΔH^+ and nocturnal CO_2 fixation has been well documented (Kluge and Ting 1978). Evaporative demand may have played a role on the ΔH^+ attained by plants. Nevertheless, since the 725-m-high location faces leeward, but the two other locations face windward, receive air from the northeastern trade winds with a higher RH, and the higher locations are cloudy most of the year (Huber 1986), RH does not help explain the differences in ΔH^+ between the 1,150- and the 1,400-m-high populations. Nor does RH contribute to understand the differences in maximum ΔH^+ found in greenhouse experiments. Detailed measurements of environmental variables could pinpoint which enhance CAM operation in *P. floccosa*.

In *P. floccosa*, 51% of the variation in ΔH^+ was explained by seasonal changes in TR_{int} among experiments. Values of ΔH^+ were 1.4 times as large at 4.3 than at 3.0 kW m^{-2} d^{-1} TR_{int} (approximately 36 and 25 mol m^{-2} d^{-1} photosynthetic photon flux density, respectively, on a clear day in Caracas). In several species of agaves and cacti, total incident photosynthetic photon flux density up to approximately 20 mol m^{-2} d^{-1} increased ΔH^+ and this observation was explained based on the need of CAM operation for glucans synthesized during the day (Nobel 1988).

We conclude that *P. floccosa* is capable of CAM activity in the C_3-CAM mode, with a large variability among populations and drought experiments, and that CAM increases with water deficit. Elevation does not seem to bear an influence on values of $\delta^{13}C$. The variability observed was partly explained in drought experiments by seasonal changes in incident radiation.

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