Chapter 4

CAM Photosynthesis in Bromeliads and Agaves: What Can We Learn from These Plants?

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1. Introduction

The term Crassulacean Acid Metabolism (CAM) was introduced in the 1940s as a result of observations in *Bryophyllum calycinum*, a crassulacean plant, which showed prominent diel variations in leaf acid content, with increases at night followed by daytime deacidification [1, 2]. Nowadays, we know that CAM is present in plant families other than Crassulaceae, being found in about 20,000 terrestrial and aquatic species, with representatives in 343 genera of 35 families [3, 4].

In general, CAM photosynthesis consists of the nocturnal carboxylation of phosphoenolpyruvate (PEP) by using atmospheric or respiratory CO_2, giving rise to oxaloacetate (OAA), a reaction mediated by the enzyme phosphoenolpyruvate carboxylase (PEPC). OAA is then reduced by malate dehydrogenase (MDH) to malate, which is subsequently transported into the vacuole and stocked in the form of malic acid, generating the typical nocturnal acidification of CAM plants. This transport into the vacuole is mediated by an active process of proton pumping through H^+-V-ATPases in the tonoplast and an organic acid anion channel. In the following light period, the stomata are maintained closed, and vacuolar malic acid is remobilized into the cytoplasm (returning to the malate form) and decarboxylated, releasing CO_2 (a process mediated by malic enzyme, ME-type, or phosphoenolpyruvate carboxykinase, PEPCK-type, enzymes, depending on the plant species) and causing the deacidification of the cells. The liberated CO_2 is refixed in the chloroplasts by the bifunctional enzyme ribulose-bisphostate carboxylase/oxygenase (Rubisco) [4-8].

Given the complex set of overlapping phenomena in CAM plants, including the diel patterns of acid synthesis and degradation, the presence of two carboxylases (PEPC and Rubisco) and the peculiar stomatal opening behaviors, the concept of CAM phases was proposed, for didactic purposes. Basically, CAM can be divided into four phases: 1) Phase I consists of the nocturnal fixation of CO_2 via open stomata and its storage in the form of organic acids in the vacuole; 2) at the start of Phase II in the early morning, when PEPC is becoming inactive while Rubisco is progressively being activated, the stomata still remain open, and the fixation of CO_2 can occur via both enzymes; 3) during Phase III, the stomata remain closed, while the organic acids are remobilized from the vacuoles and decarboxylated, generating CO_2 to Rubisco in the Calvin-cycle (C_3); and finally 4) in Phase IV, which occurs at the transition from the light to the dark period, the storage of organic acid is already exhausted and the stomata reopen again, allowing the CO_2 to be assimilated directly in carbohydrates via the Calvin-cycle [8-11] (Figure 1).

Considering the fact that CAM photosynthesis continuously offers CO_2 to Rubisco (during Phases II and III), in essence, this photosynthetic adaptation consists of a CO_2-concentrating mechanism. The internal storage of carbon in the form of organic acids and its subsequent decarboxylation generates an internal increase of CO_2, which largely reduces the oxygenase activity of Rubisco, therefore alleviating photorespiration [12]. In fact, reduced photorespiratory rates in CAM plants are expected to occur mainly during the first part of the light period when prominent organic acid decarboxylation fluxes significantly elevate internal CO_2 concentration. On the other hand, depending on the duration of the light period, the photosynthetic active radiation (PAR) received and the amount of organic acid accumulated during the previous night, CAM plants might also face the completely opposite scenario in terms of internal CO_2 and O_2 availability during the last part of Phase III since the eventual depletion of organic acid reserves and the daytime accumulation of O_2 produced due to the photosynthetically-driven water photolysis behind closed stomata would lead to O_2/CO_2 rates greatly favoring the oxygenase activity of Rubisco. In addition, under severe water stress conditions, stomatal opening at Phase IV might not occur, leading to high levels of photorespiration during the last hours of the light period [12].

In a similar way to CAM, C_4 photosynthesis also represents an important CO_2-concentrating mechanism for terrestrial plants. Although sharing many biochemical similarities and evolutionary driving forces, C_4 and CAM also display marked differences. For instance, C_4 plants spatially concentrate a small pool of transitory C_4 acids in mesophyll cells that turnover rapidly in the bundle sheath cells, while the concentrating mechanism of CAM plants is based on the temporal storage of a larger pool of end products of C_4 acids (mainly malic acid) in the vacuoles at night, which slowly turnover in the cytoplasm of the same photosynthetic cells during the day [3]. Another significant difference between C_4 and CAM photosynthesis involves the mechanism used to separate the two carboxylase activities, *i.e.*, a spatial PEPC/Rubisco separation in C_4 (PEPC in the mesophyll and Rubisco in the bundle sheath cells) and a temporal separation between these enzymes in CAM plants (PEPC at night and Rubisco during the day). C_4 and CAM are not only advantageous CO_2 concentrating systems but are also mechanisms capable of providing elevated water use efficiency (WUE). In C_4, the

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concentration of CO_2 at the site of Rubisco activity usually reduces stomatal conductance and, therefore, the general transpirational water losses through the light period. On the other hand, the ability to restrict stomatal opening during the periods in which higher air humidity is available (*e.g.*, at night, during dawn and dusk) allows CAM plants to fix higher amounts of CO_2 with very low rates of water loss through transpiration.

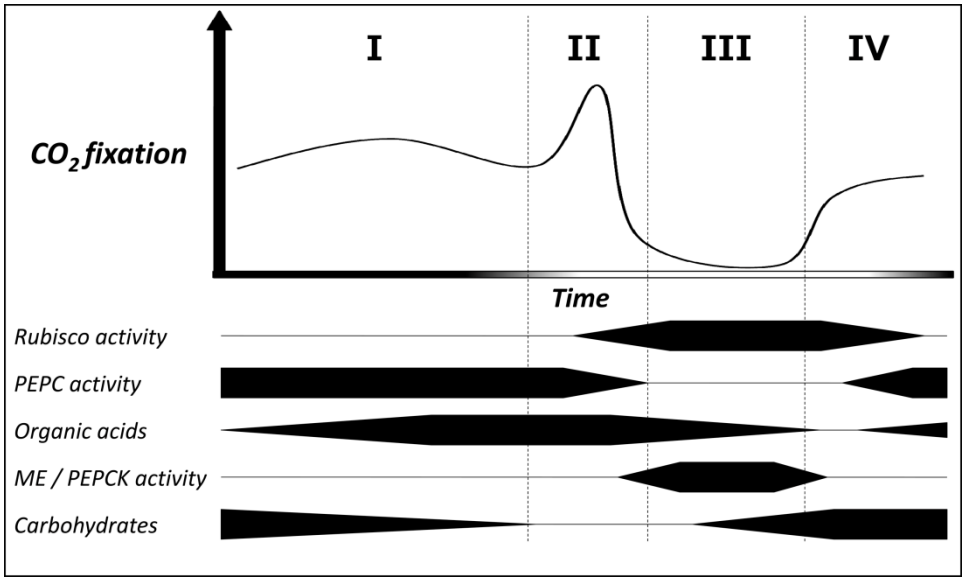

Figure 1. Four temporal Phases of CAM (I, II, III and IV) indicated over a 24-hour photoperiod by the main features of CAM: CO_2 fixation, Rubisco, PEPC and NAD(P)-ME-type or PEPCK-type activities and organic acid and carbohydrate accumulation. The black shapes below the x-axis indicate when the processes described above were happening. The black and white bars in the x-axis indicate night and day, respectively.

The presence of two pathways of carboxylation in CAM plants, one mediated by PEPC and the other by Rubisco, determines the differences in the ratio of carbon isotope discrimination ($\delta^{13}C$). Those differences are given by the fractionation characteristics of PEPC and Rubisco. Therefore, $\delta^{13}C$ values found in the plant are indicative of how much CO_2 was fixed by Rubisco (during the day) or PEPC (at night). Consequently, growth dependent on dark CO_2 fixation generates less negative values of $\delta^{13}C$ (CAM plants) while growth dependent on direct atmospheric CO_2 fixation during the day results in more negative $\delta^{13}C$ values (C_3 plants) [13]. For this reason, the values of $\delta^{13}C$ found in the tissues of plants leads to their classification as either CAM or C_3.

In the early 1970s, Winter and von Willert [14] discovered the capacity of *Mesembryanthemum crystallinum*, a plant until that time considered C_3, of expressing CAM (fixing CO_2 at night) when treated with NaCl, showing that plants did not uniquely express CAM or C_3. After this discovery, Osmond and cols. [13] analyzed the $\delta^{13}C$, CO_2 fixation and malate synthesis when

regulating day/night length and temperature during growth of *Kalanchoë daigremontiana* and *K. blossfeldiana*. The results led to the conclusion that, depending on the environmental conditions, CAM plants are capable of using both C_3 (Rubisco) and C_4 (PEPC) fixation mechanisms during their development. Thus, the environmental conditions regulate the proportion of carbon acquired through each pathway.

1.1. Types of CAM

With more information in the literature regarding CAM, we now know that not all CAM plants just fix CO_2 at night while the stomata remain closed during the day (*e.g.* classical CAM); CAM photosynthesis is now considered a more flexible phenomenon in plants. It has been proposed that there is a continuum of photosynthetic expression from C_3 to CAM and in this progression several types of CAM photosynthesis can be found, ranging from a weak to a strong degree of expression [4], which is determined by the stage of plant development, environmental conditions and/or the species. Although classical CAM was characterized as traditionally showing a four-phase pattern, the flexibility of CAM and the diversity of CAM species illustrate the existence of a wide range of responses; thus, within CAM, three main types can be identified: classical CAM, CAM-cycling and CAM-idling.

The term CAM-cycling is used to explain the photosynthetic condition represented by a diel gas exchange pattern similar to that found in C_3 plants, combined with nocturnal organic acid accumulation [7, 14, 15], which results from the internal respiratory CO_2 refixation via PEPC at night. During the day, these acids are decarboxylated, releasing CO_2 to Rubisco, while the stomata remain open [8,16]. CAM-cycling can be expressed in some species at the early stages of shifting from C_3 to CAM in response to water limitation (facultative CAM plants) [16], maintaining a positive carbon balance during repeated drought events [17], reducing respiratory CO_2 losses, allowing water retention and extending the life cycle [7]. On the other hand, CAM-idling exhibits a null CO_2 gas exchange over the entire 24-hour period, while a small nocturnal acid accumulation still continues [7,15], in which the respiratory CO_2 is recaptured and used for synthesizing organic acids at night, which are decarboxylated the following day, recovering the carbohydrate used during the refixation of respiratory CO_2 [6]. Thus, under extended drought conditions, for example, CAM-idling allows recycling internal CO_2, avoiding a negative balance of carbon at the expense of growth and maintaining photosynthetic competence [16].

1.2. Constitutive and facultative CAM

Some species possess the capacity to exhibit facultative CAM, in which the degree of CAM expression greatly varies depending on internal or environmental cues. Facultative CAM may be part of the ontogenetic plant program, in which environment factors could accelerate or delay the preprogrammed C_3-CAM transition [17-20]. Thus, it is possible to find variable degrees of CAM modulation in facultative species, ranging from exclusively environmental to strictly developmental control of CAM, passing through an intermediate state in which both environmental and developmental cues influence CAM expression [19, 20] (Figure 2). Some

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well-characterized examples of facultative CAM plants are *Mesembryanthemum crystallinum* and some *Clusia* species.

As above mentioned, *Mesembryanthemum crystallinum* was initially considered an exclusive C_3 plant; however, after the discovery of its capacity to switch from C_3 to CAM [14], this plant became a common model species for facultative CAM photosynthesis research. *M. crystallinum* is an annual halophyte widely distributed in places with hot and dry summers, wet and cold winters and increased salinity, as found in south and east Africa, along the coasts of the Mediterranean and west United States [21, 22]. Thus, after a short cold and rainy season (winter), *M. crystallinum* germinates and the juvenile plants perform exclusively C_3 photosynthesis. Then, during the summer, in which the temperature and salinity increase and water availability decreases, the mature plants produce smaller and succulent leaves with epidermal bladders coinciding with the developmental CAM induction [21, 23]. Since young plants cannot perform CAM, ontogenetic factors become visibly important for this plant [24], and stressful conditions, like high salinity, would only accelerate the rate of CAM induction already preprogrammed by the plant development [8, 9, 17, 21]. Nevertheless, Winter and Holtum [19] observed that *M. crystallinum* is capable of performing C_3 photosynthesis during its entire life cycle if maintained in non-saline and well-watered conditions, demonstrating that CAM induction in *M. crystallinum* is mainly controlled by environmental conditions rather than by preprogrammed developmental processes. Once *M. crystallinum* shifts from C_3 to CAM, it never returns to C_3 mode [17, 21, 22], even if the adverse conditions are removed. Curiously, some degree of reversibility in CAM-induced *M. crystallinum* plants has already been reported [25], but more studies are necessary to confirm this. On the other hand, some species of the genus *Clusia* clearly show their capacity to switch from C_3 to CAM and back again in response to different environmental conditions.

In fact, the genus *Clusia* represents a magnificent example of diversity and inducibility of CAM. In *Clusia minor*, for example, opposite leaves on the same node can perform either C_3 or CAM in response to different temperatures and leaf-air vapor pressures, as only the leaves maintained in dry air conditions were capable of expressing CAM [26]. Among *Clusia* species, *C. minor* is currently the most widely used model for C_3-CAM photosynthesis studies [18, 20, 27, 28]. It has already been shown that juvenile plants of *C. minor* perform C_3 photosynthesis and switch their photosynthetic behavior to CAM when mature, increasing dark CO_2 fixation [18, 20], activity of PEPC and PEPCK (key enzymes in CAM photosynthesis), and nocturnal accumulation of organic acids [18]. Thus, apparently, *C. minor* plants are controlled by programmed development. However, these plants when exposed to drought (or dry season in nature) up-regulate CAM activity [18, 20, 29, 30], indicating that CAM can also be controlled by the environment, even though reversion to the C_3 state never occurs. Therefore, both development and environment are capable of regulating CAM in *C. minor*. In another species of the same genus, *C. pratensis*, shows that, in this case, CAM responds almost exclusively to environmental cues and the switch from C_3 to CAM is fully reversible [20].

Plants that always perform CAM in mature tissues independently of the environmental conditions (stressful or not) are classified as constitutive (or obligate) CAM species. Examples of constitutive CAM are some species from the Cactaceae family (*e.g.*, some *Opuntia* species),

Crassulaceae (*e.g.*, some *Kalanchoë* species) [16, 20] and Clusiaceae (*C. rosea*) [20, 31]. However, despite being classified as constitutive CAM plants, some of these species might also show some degree of plasticity in CAM expression in response to environmental conditions (Figure 2). *Kalanchoë pinnata*, *K. daigremontiana* and *Opuntia ficus-indica*, for example, are capable of up-regulating CAM when maintained under drought conditions [16, 20, 32]. Also, *Opuntia basilaris*, another constitutive CAM species, enhances its nocturnal CO_2 fixation when exposed to favorable conditions of watering [33]. It should be noted that neither favorable nor unfavorable conditions are capable of changing the CAM mode to C_3 mode because the ontogenetic program of the plant does not allow such modification [20, 32].

Nowadays, there is an important debate going on about establishing clear differences between facultative CAM and constitutive CAM plants since it has already been demonstrated that, in some constitutive CAM plants, when juvenile (*e.g.*; *K. daigremontiana, K. pinnata, Opuntia ficus-indica* and *Clusia rosea*), C_3 photosynthesis is the main pathway to uptake CO_2 and CAM only becomes the dominant pathway when they are mature [20, 29]. For example, *C. rosea* seems to be strongly controlled by ontogenetic factors, as variations in the environment did not affect the degree of CAM in this species; nevertheless, when juvenile, facultative and fully reversible CAM can be observed in this species [20]. Thus, due to the facultative component that exists in constitutive CAM, it would be very difficult to strictly define a species as facultative or constitutive CAM. We, therefore, propose that facultative CAM species should be those that are capable of going from typically C_3 photosynthesis to CAM and back, even in adulthood. However, species that are capable of expressing CAM at some moment in their life cycle, regardless of whether they are influenced by either environmental or developmental factors, but cannot return to a C_3 after they become adults should be considered simply CAM plants.

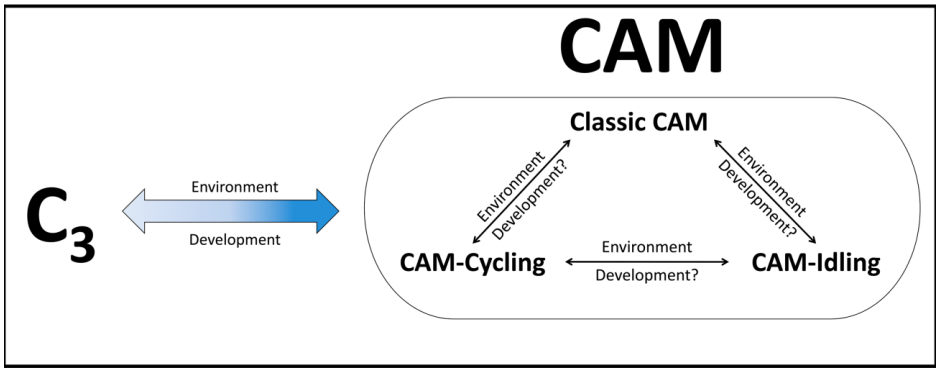

Figure 2. Capacity of plants to transition from C_3 to CAM modes is controlled by environmental and developmental cues (blue arrow). CAM and its flexibility to transit between different modes of CAM (classic-CAM, CAM-cycling and CAM-idling) is under environmental control; however, it is unknown if it could also be regulated by developmental factors.

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2. Biochemistry of CAM

2.1. Importance of carbohydrates in CAM plants

Due to its biochemical nature, the CAM cycle is inexorably associated with a serious metabolic constraint: at the end of the Phase IV (end of the light period – Figure 1) there must be an adequate pool of carbohydrates to generate PEP, required for nocturnal CO_2 fixation via PEPC during Phase I. That pool of carbohydrates must differ from the pool of carbohydrates needed to ensure other metabolic processes including dark respiration, export and growth [6, 9, 17, 34, 35]. In several CAM species, the net flux of carbon for regenerating PEP is mainly based on the pool of starch; thus, these plants show a large diel change in transitory starch [34, 36, 37]. Therefore, when CAM is induced, an increase in starch degradative enzymes is required [38, 39], and specific transporters of intermediate products across the chloroplast membrane must also be present [38, 40]. The degradation of transitory starch can be very important in CAM plants providing PEP through glycolysis, as demonstrated for *Mesembryanthemum crystallinum,* in which mutant plants deficient in starch synthesis due to a lack of the enzyme phosphoglucomutase were incapable of operating in CAM mode and had lower fecundity than the wild type. However, when these mutants were fed with glucose, the nocturnal acidification was recovered [41].

Interestingly, it was observed that *M. crystallinum*, when switching from C_3 to CAM, changes the metabolic pathway of starch degradation from hydrolytic to phosphorolytic [42]. In the latter, after the starch is degraded, the main product exported from the chloroplast is glucose-6-phosphate, while in the hydrolytic pathway the main product exported is maltose [43]. Thus, when in C_3 mode, *M. crystallinum* exports maltose from the chloroplast as a result of starch degradation [42], but when it operates in CAM mode, the export switches from maltose to glucose-6-phosphate [42, 44]. Interestingly, it was observed that in several so-called facultative CAM species exposed to drought or salinity stress, PEPC increased its sensitivity to activation by glucose-6-phosphate [45, 46]. This may suggest a link between the metabolic pathway of phosphorolytic starch degradation and PEPC activation. It has been proposed that starch degradation and its flux through PEPC is under circadian regulation in CAM plants because it was observed that the activity of some enzymes that participate in the starch degradation (*e.g.* β-amylase and starch phosphorylase) are coordinated in time with the phosphorylation of PEPC at night [9].

As mentioned above, transitory starch plays an important role in the transition from C_3 to CAM in plants of *Mesembryanthemum crystallinum* [41] because starch is the main carbohydrate used to generate PEP either in primary or axillary leaves [34]. The partitioning of assimilates in different pools originating from C_3-carboxylation or C_4-carboxylation have been suggested as a possible regulatory mechanism of carbohydrate metabolism in CAM plants [47]. It has also been demonstrated that this carbohydrate partitioning has a crucial ecophysiological function besides allowing CAM to function; in C_3 primary leaves of *M. crystallinum*, the carbohydrate partitioning works mainly to facilitate the development of the whole plant. Then, when the dry season arrives, the growth of axillary leaves accelerates and the development of the plant is mainly directed towards reproduction. Therefore, when the plant switches from C_3 to CAM,

the axillary leaves are capable of exporting sugars during the day and the night derived from C_3 and C_4-carboxylation, in contrast to primary leaves, which possess a limited export of sugars [34]. Thus, the axillary leaves ensure the reproductive success by exporting sugars, while starch guarantees the CAM cycle [17, 34]. Interestingly, measurements of $\delta^{13}C$ in seeds of this species showed a value of -16.4‰, indicating an important contribution of the nocturnal CO_2 fixated (CAM) to seedset [48].

Although starch represents the main carbohydrate to provide PEP in many CAM plants, there are also CAM plants that show a smaller diel change in starch levels because they are also capable of storing carbohydrates in the form of hexose inside the vacuole [49], as observed in *Ananas comosus* (pineapple) [50, 51]. Therefore, there are differences among CAM plants in their diel changes of energy-rich compounds used for nocturnal organic acid synthesis [52].

In addition, it was observed that species of *Clusia* display differences between their carbohydrate pools derived from C_3 and C_4-carboxylation and the partitioning of each pool to storage or exportation. In *Clusia minor*, in which CAM expression is environmentally and ontogenetically controlled, there was a doubling of its pool of carbohydrates after the switch from C_3 to CAM. Interestingly, two pools of soluble sugars were identified, one enriched in ^{13}C and the other depleted in ^{13}C, the latter destined to be transported, indicating the existence of a regulated partitioning of carbohydrates in this species [47]. Surveys comparing *Clusia minor* (performing CAM) and *Clusia rosea* (constitutive CAM) showed that the carbohydrate pool in leaves of *C. rosea* is mainly derived from PEPC carboxylation, while the carbohydrate pool of *C. minor* is derived from Rubisco carboxylation. In both species, leaf soluble sugars were enriched in ^{13}C when compared with the leaf starch, indicating that C_3 assimilates were preferably redirected into starch [34]. In addition, in the same work, it was demonstrated that regardless of the degree of CAM the pool of carbohydrates exported from the leaves to reproductive sinks (fruits) was mainly derived from C_3-carboxylation (showing values of $\delta^{13}C$ of -25.6 and -25.8 in *Clusia minor* and *Clusia rosea*, respectively). Thus, those results confirm the existence of different partitioning of assimilates derived from C_3 or C_4-carboxylation between reproductive and vegetative growth. As observed for most CAM plants, growth and productivity are maximized when direct CO_2 fixation via Rubisco in Phase IV predominates [17, 34, 35]. Therefore, the fact that assimilates are formed during this phase and exported from the leaves to the sink shows the importance of Rubisco in the reproductive success and growth of CAM plants.

2.2. An overview of PEPC and PPCK regulation

PEPC is a homotetrameric enzyme that participates in a broad range of functions (both photosynthetic and non-photosynthetic) in the plant cell [53], not found in fungi nor animals [54]. In C_4 and CAM plants, PEPC irreversibly carboxylates PEP, using HCO_3^- and generating oxaloacetate (OAA). Both the capacity to use HCO_3^- instead of gaseous CO_2 (different from Rubisco) and the high affinity for HCO_3^- make CAM plants more efficient in terms of nitrogen usage because less nitrogen allocation is required to form adequate quantities of the carboxylating enzymes (PEPC and Rubisco) than in C_3 plants, in which a larger pool of Rubisco is required to fixate CO_2.

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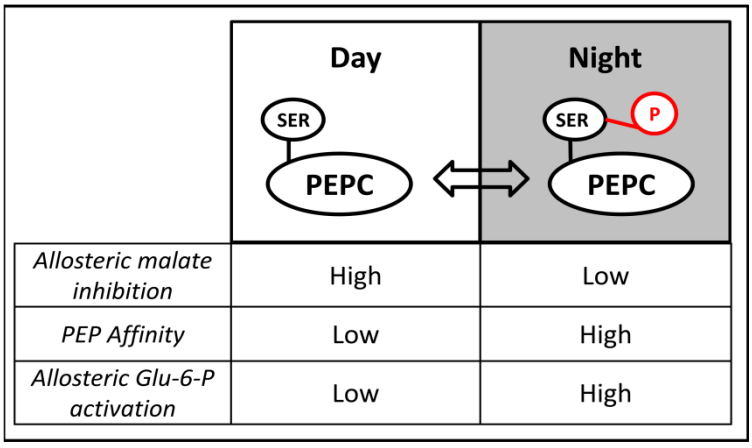
Due to the activity of two carboxylase enzymes (PEPC and Rubisco) temporally separated in CAM plants, a tight metabolic control to avoid futile cycles of carbon must exist [6]. PEPC enzyme can be regulated by environment and endogenous circadian signals, resulting in the reversible activation of the enzyme by phosphorylation. This regulation of PEPC through phosphorylation is mediated by a PEPC kinase enzyme (PPCK- a specific Ca^{2+} -independent serine/threonine kinase), which phosphorylates PEPC on its serine residue (Figure 3), reducing its sensitivity to malate inhibition at night [55] and increasing its allosteric activation by glucose 6-phosphate and affinity for PEP [53]. During the day, PEPC is highly inhibited by malate because this enzyme is dephosphorylated. Thus, PEPC activity is restricted to Phase I, early Phase II and late Phase IV [6, 55] (Figure 1), minimizing the futile cycling of simultaneous malate synthesis (mainly at night) and breakdown (only during daytime).



	Day	Night
Allosteric malate inhibition	High	Low
PEP Affinity	Low	High
Allosteric Glu-6-P activation	Low	High

Figure 3. Daytime inactivation and nighttime posttranslational activation of PEPC enzyme. Activation of PEPC at night depends on the phosphorylation of its serine residue by PPCK enzyme. Different allosteric malate inhibition and Glu-6-P activation and affinity for PEP in light and dark periods is shown.

PEPC kinase enzyme is mainly regulated by transcriptional level abundance, showing the peculiar characteristic among kinase enzymes of requiring *de novo* synthesis to be active [55]. In C_3 and C_4 plants, PPCK seems to be activated by light [56-59], while in CAM plants this enzyme responds mostly to a circadian oscillator and is active during the night [55, 60, 62]. Taybi and cols. [63] showed that the transcript accumulation of PPCK in *M. crystallinum* is largely subjected to circadian control under continuous light condition. However, that circadian control is itself regulated by other factors, such as cytosolic malate level [55, 64]. In *Zea mays*, a C_4 species, it was found that ZmPPCK2, a specific PPCK isoform, is expressed preferentially during the night [59]. These findings point toward a flexibility in the expression

of this enzyme and indicate that a simple step could lead to its expression during nighttime, as happens in CAM plants.

Differences in regulation of PEPC would also require changes in Rubisco activity to avoid futile cycling of CO_2. In a C_3 plant, Rubisco apparently needs to be activated by Rubisco activase (RCA), which responds positively to irradiance and ATP supply and negatively to high concentrations of sugar phosphate [65]. The same authors found that in CAM-induced *M. crystallinum*, the activation of Rubisco followed a rhythmicity of activation-deactivation. Whether this is due to circadian or metabolite control, the authors could not confirm.

3. CAM origins

Phylogenetic reconstructions from comparative physiology and taxonomy confirm that C_3 photosynthesis is ancestral to both CAM and C_4, with CAM appearing multiple times in the taxa and earlier than C_4 photosynthesis [54, 66]. Since all enzymes related to CAM functions are also found in C_3 plants, several aspects of CAM appear to have evolved as minor modifications of processes already present in ancestral C_3, suggesting that CAM may have originated from the reorganization of ancient metabolic pathways (co-option) [67]. Thus, processes such as gene duplication, processing of mRNA and gene expression control by *cis*-regulatory elements or enhancers can maintain essential ancestral functions along with new ones related to CAM [67].

The majority of CAM plants is found in monocot and eudicot taxa, which had high diversification by the early and middle Miocene [68]. It has been hypothesized that atmospheric CO_2 levels and the CO_2/O_2 ratio decreased sufficiently some time after the Cretaceous [69] and during the Miocene, allowing the evolution of CAM in terrestrial and aquatic environments [66, 70]. Thus, atmospheric CO_2 reductions, coupled with warm climates in subtropical latitudes, were factors with negative impacts on C_3 photosynthesis, due to a depletion of CO_2 diffusion gradient that resulted in a decrease in the carboxylation efficiency and an increase in photorespiration rates [71]. Therefore, the restricted daytime CO_2 availability in that environment may have been the most important driving force in the evolution of CAM. As a result, plants that rely on the C_4 and CAM pathway would have advantages compared to C_3 plants because of the higher carboxylation efficiency [70]. It is also proposed that a photosynthetic mechanism similar to aquatic CAM arose during the late Paleozoic, also a period of low atmospheric CO_2 concentrations [72]. Thus, CAM photosynthesis may have emerged several times as a means of improving carbon economy.

Currently, another evidence of the multiple origins of CAM is its occurrence in 35 taxonomically diverse families [16], of which 32 belong to Magnoliophyta division. Among these 32 families, eight are monocots (Agavaceae, Alismataceae, Araceae, Asphodelaceae, Bromeliaceae, Hydrocharitaceae, Orchidaceae and Ruscaceae) [4].

Nowadays, there is little fossil evidence about CAM origins. *Isoetes* species are at present-day a monophyletic CAM taxon which represents the oldest clade of known CAM plants since

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there is fossil evidence of the existence of that taxon by the early Cretaceous [70]. More recent CAM plant fossils are found from the middle Miocene and late Pleistocene to the Holocene. The plant fossil from the middle Miocene, *Protoyucca shadishii*, is very likely an early member of dry communities of present *Yucca* CAM plants (Agavaceae) [73]. Another survey analyzed $\delta^{13}C$ values of samples of CAM plant *Opuntia polyacantha* (Cactaceae) found in old pack rat middens in the southwestern United States more than 40,000 years old and others 10,000 years old, in which a shift in the $\delta^{13}C$ value from -21.9‰ (in the 40,000-year-old sample) to - 13.9 ‰ (10,000-year-old sample) was observed. These results provided physiological evidence of drier climates in the late Pleistocene in that region [74], which apparently favored CAM expression. Recently, the fossil *Karatophyllum bromelioides* from the late Pleistocene to Holocene was studied, which shares excellently correlated morphological characteristics with *Aechmea magdalenae*, a CAM bromeliad [75]. Since succulence within Bromeliaceae seems to be related to CAM (leaf thickness values greater than 1 mm showed carbon-isotope ratios lower than -20‰ [76], both the similar morphology of this fossil plant to *A. magdalenae* and the leaf thickness of 1.6 mm showed that, indeed, *Karatophyllum bromelioides* could be a fossil CAM [75].

4. CAM in Bromeliaceae

Bromeliaceae is considered a monophyletic family inside the order Poales [77]. It is one of the largest and most widespread plant families in the neotropics, occupying a wide variety of niches with different conditions [68, 78]. Recently, of the three classic subfamilies, only Bromelioideae and Tillandsioideae are considered monophyletic, while Pitcairnioideae was subdivided into five new subfamilies: Pitcairnioideae, Puyoideae, Navioideae, Hechtioideae, Lindmanioideae and Brocchinioideae [79].

In the Bromeliaceae, CAM has arisen multiple times in a seemingly independent way. Crayn and cols. [68] identified at least three independent origins for CAM in the 51 species analyzed, and this pattern was maintained when more species were taken into account. Silvera and cols. [4] also found multiple origins for CAM in the Orchidaceae family, which appeared to be linked with the colonization of the epiphytic habitat. In contrast to the Orchidaceae family, however, among bromeliads a strong correlation was not found between the occurrence of CAM and epiphytism [68]. In fact, a more recent work suggested that CAM could be more common in terrestrial, rather than epiphytic, bromeliad species [80]. Although very enlightening, these studies focused on the phylogeny and took into account only whether the plants were epiphytes or not. Some extra details could arise by observing how each species couples with its environment. For example, one of the major morphologic features of bromeliads is the rosette conformation of the leaves. This morphology is very important for some species in which the overlapping of its leaves forms a structure capable of accumulating water and organic matter – the so-called tank-bromeliads [78]. Based on the presence of the tank and how the plant acquires nutrients, Pittendrigh [81] separates bromeliads into four classes (Types I, II, III and IV - Figure 4). Table 1 shows a list of species grouped by type with their respective habitat (simplified only as moist or dry) and photosynthetic pathway.

4.1. Type I Bromeliads

Type I bromeliads are the ones that obtain their nutrients from the soil through the roots. The main differentiating feature of Type I bromeliads is that they do not have a structure capable of accumulating water. For this reason it would be expected that, when growing in dry habitats, these species would need other mechanisms to preserve water, such as CAM. Among this group are a few species that have already been studied regarding CAM. Cristopher and Holtum [50] studied some Type I bromeliads, such as *Pitcairnia paniculata*, *Fosterella schidosperma*, *Cryptanthus zonatus*, *Ortophytum vagans* and *Dyckia sp*, along with species belonging to other types. All Type I bromeliads showed some degree of CAM, except *P. paniculata*, indicating that this particular species might be better adapted to water-abundant environments, while the others may be more successful in drier habitats. In the same paper, the authors detailed the carbohydrate profile for these species but found no correlation between the type of accumulated carbohydrate and habit or CAM expression. Among the subfamilies, however, there were some similarities; for example, Tillandsioideae and Bromelioideae species accumulated starch, while plants belonging to the former Pitcairnioideae subfamily accumulated mainly soluble sugars.

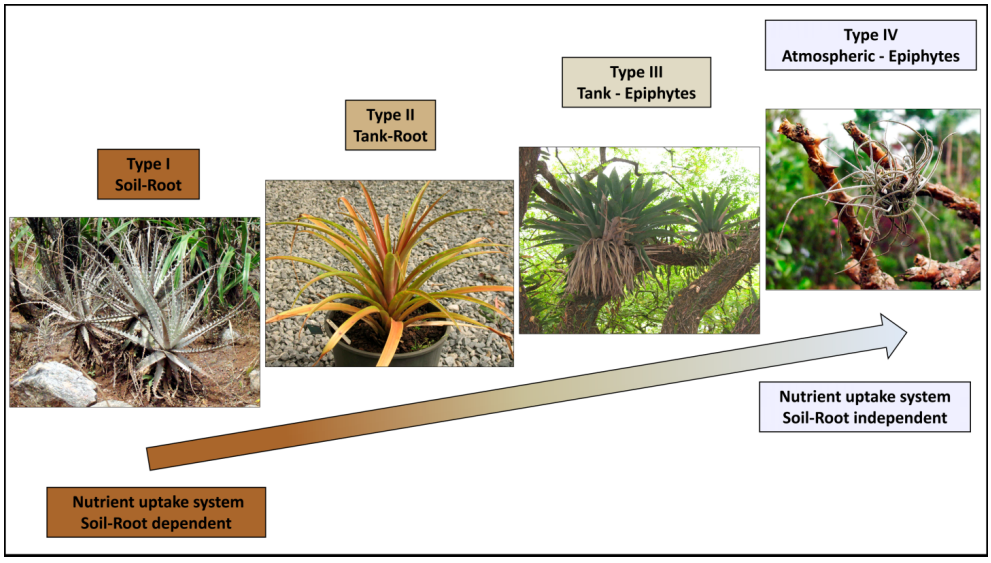

Figure 4. Four main bromeliad types proposed by Pittendrigh [81] and their dependency to acquire nutrients from the soil through a root system (upward arrow). As examples, types are illustrated by different bromeliad species. Type I –

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Soil-Root: *Dyckia* sp.; Type II - Tank-Root: *Ananas* sp.; Type III –Tank-Epiphyte: *Vriesea* sp.; Type IV – Atmospheric-Epiphyte: *Tillandsia* sp.

Species	Type	Habitat	Photosynthetic patway	Reference
Aechmea allenii	III	moist	CAM[1,2]	[76]
Aechmea dactylina	III	moist	CAM[1,2]	[76]
Aechmea fasciata	III	dry	CAM[3]	[82]
Aechmea fendleri	III	moist	CAM[2]	[83]
Aechmea floribunda	III	dry	CAM[3]	[82]
Aechmea haltonii	III	moist	CAM[3]	[68]
Aechmea magdalenae	I	moist	CAM[1]	[84]
Aechmea nudicaulis	III	dry	CAM[3]	[82]
Aechmea sphaerocephala	III	dry	CAM[3]	[82]
Ananas ananassoides	I	dry	CAM[1,2,3]	[68, 85]
Ananas comosus	II	dry	CAM[1,2,3]	[50, 85]
Araeococcus pectinatus	III	moist	CAM[3]	[68]
Billbergia amoena	III	dry	CAM[3]	[82]
Billbergia pyramidalis	III	dry	CAM[3]	[82]
Brocchinia acuminata	I	moist	C3[3]	[68, 86]
Brocchinia micrantha	I	moist	C3[3]	[68, 86]
Bromelia chrysantha	II	dry	CAM[3]	[68]
Bromelia humilis	II	dry	CAM[1,2,3]	[83, 87-88]
Bromelia plumieri	I	dry	CAM[1]	[84]
Catopsis floribunda	III	moist	C3[3]	[68]
Catopsis wangerinii	III	moist?	C3[3]	[68]
Catopsis micrantha	III	moist	C3[1,2]	[76]
Cottendorfia florida	I	dry	C3[3]	[68]
Cryptanthus zonatus	I	dry	CAM[2]	[50]
Cryptanthus beuckeri	I	dry	CAM[3]	[68]
Deuterocohnia meziana	I	dry	CAM[3]	[68, 86]
Deuterocohnia longipetala	I	dry	CAM[3]	[68, 86]
Deuterocohnia lotteae	I	dry	CAM[3]	[68, 86]
Dyckia dawsonii	I	dry	CAM[3]	[86]
Dyckia ferox	I	dry	CAM[3]	[68, 86]

Dyckia hilaireana	I	dry	CAM[3]	[68]
Dyckia sp.	I	dry	CAM[2]	[50]
Encholirium inerme	I	dry	CAM[3]	[68, 86]
Encholirium irwinii	I	dry	CAM[3]	[68, 86]
Edmundoa perplexa	III	moist	CAM[3]	[68]
Fosterella schidosperma	I	dry	CAM[2]	[50]
Fosterella elata	I	moist	C3[3]	[68, 86]
Fosterella penduliflora	I	moist	C3[3]	[68, 86]
Fosterella petiolata	I	moist?	C3[3]	[68, 86]
Guzmania calamifolia	I	moist	C3[1,2]	[76]
Guzmania circinnata	III	moist	C3[1,2]	[76]
Guzmania coriostachya	III	moist	C3[1,2]	[76]
Guzmania desautelsii	III	moist	C3[1,2]	[76]
Guzmania filiorum	III	moist	C3[1,2]	[76]
Guzmania glomerata	III	moist	C3[1,2]	[76]
Guzmania lingulata	III	moist	C3[1,2,3]	[76, 89]
Guzmania macropoda	III	moist	C3[1,2]	[76]
Guzmania monostachia	III	moist	C3/CAM[1,2,3]	[68,89-90]
Guzmania mucronata	III	moist	C3[2]	[83]
Guzmania musaica	III	moist	C3[1,2]	[76]
Guzmania scherzeriana	III	moist	C3[1,2]	[76]
Guzmania sprucei	III	moist	C3[1,2]	[76]
Guzmania subcorymbosa	III	moist	C3[1,2]	[76]
Guzmania wittmackii	III	moist	C3[3]	[68]
Hechtia glabra	I	dry	CAM[3]	[68, 86]
Hechtia glomerata	I	dry	CAM[3]	[68, 86]
Hechtia guatemalensis	I	dry	CAM[3]	[68, 86]
Hechtia lindmanioides	I	dry	CAM[3]	[68, 86]
Lymania alvimii	III	moist	CAM[3]	[68]
Mezobromelia pleiosticha	III	moist?	C3[3]	[68]
Navia igneosicola	I	moist	C3[3]	[68, 86]
Navia phelpsiae	I	moist	C3[3]	[68]
Neoregelia eltoniana	III	dry	CAM[3]	[82]
Neoregelia pineliana	III	moist	CAM[3]	[68]

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Neoregelia spectabilis	III	moist	CAM[2]	[50]
Nidularium bilbergioides	III	moist	CAM[2]	[50]
Orthophytum vagans	I	moist	CAM[2]	[50]
Pepinia beachiae	I	-	C3[3]	[68, 86]
Pitcairnia arcuata	I	moist	C3[1,2]	[76]
Pitcairnia burle-marxii	I	moist	C3[3]	[68]
Pitcairnia carinata	I	moist	C3[3]?	[68]
Pitcairnia corallina	I	moist	C3[3]	[86]
Pitcairnia heterophylla	I	moist	C3[3]	[68, 86]
Pitcairnia hirtzii	I	moist	C3[3]	[68]
Pitcairnia orchidifolia	I	moist	C3[3]	[68,86]
Pitcairnia paniculata	I	moist	C3[2]	[50]
Pitcairnia poortmanii	I	moist	C3[3]	[68]
Pitcairnia recurvata	I	moist	C3[3]	[68, 86]
Pitcairnia rubronigriflora	I	-	C3[3]	[68, 86]
Pitcairnia squarrosa	I	moist	C3[3]	[68, 86]
Pitcairnia smithiorum	I	moist	C3[3]	[86]
Pitcairnia sprucei	I	moist	C3[3]	[86]
Pitcairnia valerii	I	moist	C3[1,2]	[76]
Pitcairnia wendlandii	I	moist	C3[3]	[68]
Portea petropolitana	I	moist	CAM[2]	[50]
Puya aequatorialis	I	dry	C3/CAM[3]	[68, 86]
Puya floccosa	I	dry	C3/CAM[2,3]	[92]
Puya humilis	I	dry	C3/CAM[3]	[68, 86]
Puya laxa	I	dry	C3/CAM[3]	[68, 86]
Puya werdermannii	I	dry	C3/CAM[3]	[86]
Racinaea fraseri	III	moist	C3[3]	[68]
Racinaea spiculosa	III	moist	C3[1,2]	[76]
Ronnbergia explodens	I	moist	C3[1,2]	[76]
Tillandsia anceps	III	moist	C3[1,2]	[76]
Tillandsia balbisiana	IV	moist	CAM[1,2]	[93]
Tillandsia bulbosa	IV	moist	CAM[1,2,3]	[76]
Tillandsia circinnata	IV	moist	CAM[1,2]	[93]
Tillandsia dodsonii	III	moist	C3[3]	[68]

Tillandsia fasciculata	III	moist	CAM[1,2]	[93]
Tillandsia fendleri	III	moist	C3[2]	[83]
Tillandsia flexuosa	III	dry	CAM[2]	[83]
Tillandsia gardneri	IV	dry	CAM[3]	[82]
Tillandsia ionantha	IV	moist	CAM[1,2]	[94]
Tillandsia monadelpha	III	moist	C3[1,2]	[76]
Tillandsia pohliana	IV	moist	CAM[1,2]	[91]
Tillandsia recurvata	IV	moist	CAM[1,2]	[93]
Tillandsia schiedeana	IV	moist	CAM[1,2]	[93, 95]
Tillandsia setacea	IV	moist	CAM[1,2]	[93]
Tillandsia stricta	IV	dry	CAM[3]	[82]
Tillandsia tricolor	IV	moist	CAM[2]	[50]
Tillandsia usneoides	IV	moist	CAM[1,2,3]	[82, 93, 96]
Tillandsia utriculata	III	moist	CAM[1,2]	[93, 97]
Tillandsia valenzuelana	III	moist	CAM[1,2]	[93]
Vriesea carinata	III	moist	C3[2]	[50]
Vriesea espinosae	III?	moist?	CAM[3]	[68]
Vriesea espinosae	III?	moist?	CAM[3]	[68]
Vriesea monstrum	III	moist	C3[1,2]	[76]
Vriesea procera	III	dry	C3[3]	[82]
Vriesea sucrei	III	dry	CAM[3]	[82]
Werauhia viridifolia	III	moist	C3[3]	[68]
Werauhia capitata	III	moist	C3[1,2]	[76]
Werauhia greenbergii	III	moist	C3[1,2]	[76]
Werauhia hygrometrica	III	moist	C3[1,2]	[76]
Werauhia jenii	III	moist	C3[1,2]	[76]
Werauhia kupperiana	III	moist	C3[1,2]	[76]
Werauhia lutheri	III	moist	C3[1,2]	[76]
Werauhia milennia	III	moist	C3[1,2]	[76]
Werauhia panamensis	III	moist	C3[1,2]	[76]
Werauhia vittata	III	moist	C3[1,2]	[76]

Table 1. Habitat (moist or dry), type classification (according to Pittendrigh [81]) and photosynthetic pathway of 129 bromeliad species. Photosynthetic pathways were defined by [1] Gas exchange, [2] Biochemical parameters, and [3] $\delta^{13}C$ values.

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4.2. Type II Bromeliads

The leaves of Type II bromeliads are disposed in such a way that their bases overlap, forming a space where water and nutrients can accumulate, called "tank". The absorption of the contents inside the tank can be performed in some cases by a root system that grows through the overlapping rosette leaves (called tank-roots) or even directly by the leaves, through epidermal structures called absorbing trichomes [78]. In this group, however, absorption of water and nutrients is still mostly performed by the roots. Some studies regarding CAM have been performed in this type of bromeliad. For instance, in *Bromelia humilis*, Medina and cols. [87] found differences in nocturnal CO_2 fixation and acid accumulation that increased in the wet season and with irrigation. Similar results were reported by Lee and cols. [98], indicating that in this possibly constitutive CAM plant, a more favorable condition would lead to higher stomatal conductance during the night, thereby increasing acid accumulation and plant growth. Partially confirming this, Fetene and cols. [88] showed more nocturnal CO_2 fixation by *Bromelia humilis* in the presence of nitrogen and higher irradiance. On the other hand, it was also demonstrated that another species, *Puya floccosa*, is capable of increasing nocturnal acid accumulation in response to drought and/or other unfavorable microclimatic cues [92]. Working with *Achmea magdalenae*, *Bromelia plumieri* and *Ananas comosus*, Skillman and cols. [84] showed that these plants performed well in a shaded understory, presenting higher photosynthetic capacity when compared to C_3 plants growing in the same conditions. The growth rate, however, was inferior, when the same comparison was made, possibly because of differences in the partitioning of carbohydrates produced. Also in this work, it was noted that CAM plants grew more during the dry season, different from the C_3 plants, which grew more in the wet season. Therefore, CAM seems to be advantageous over C_3 photosynthesis in conditions with water shortage. This can be easily observed in facultative CAM species, in which CAM is often induced by drought.

Recent studies on *Ananas comosus* provided some insight into the signaling of CAM up-regulation in the Bromeliaceae [99]. Although considered a constitutive CAM species, this work indicated that *A. comosus* is capable of performing C_3 photosynthesis when young plants are grown under *in vitro* conditions. In this same study, by investigating the signaling events controlling pineapple CAM expression in response to water deficit, the authors characterized the existence of at least three signaling pathways: one inhibitory, mediated by cytokinins, and two stimulatory, one dependent and one independent of ABA. Furthermore, both stimulatory pathways converged on cytosolic calcium signaling, while the ABA-dependent pathway also involved the free radical nitric oxide. Another intriguing observation was made on *A. comosus*, along with other species, regarding the longitudinal distribution of metabolites along the leaves. Popp and cols. [83] showed that in this species, along with six other bromeliads, the nocturnal accumulation of acids and the amount of carbohydrates showed an increase from the leaf base to the tip. This interesting longitudinal gradient along the leaves of bromeliads, and possibly other rosette plants, will be addressed and further discussed later in this chapter.

Apparently, CAM occurs in Type I and Type II bromeliads (both terrestrial) when required by the environment (*e.g*, dry or exposed habitats). Types III and IV, however, are epiphytic.

4.3. Type III Bromeliads

Epiphytes notably enrich the tropical forest ecosystems by providing new niches for a great number of organisms [100, 101] but are subjected to several environmental limitations. For example, nutrients and water are only available sporadically or seasonally through rainfall [102]. Therefore, epiphytes may face water shortage even if living in a moist environment like tropical forests.

One of the main characteristics of Type III bromeliads is the presence of the tank. In these plants, the acquisition of water and nutrients comes mainly from the solution impounded there [78]. These species present a large number of absorptive trichomes on the base of the leaves, which allow them to directly absorb water and nutrients from the tank [78]. Their roots play a minor role in nutrition, being more restricted to mechanical support [103]. In the epiphytic habitat the tank is a very important structure, which allows the accumulation of water and/or nutrients during drier periods. Therefore, Type III bromeliads have a reservoir of water even when rain is absent. Another remarkable feature of epiphytic tank bromeliads is their interaction with other organisms. The rosette conformation provides different compartments with distinct ecological conditions, serving as favorable habitats for a wide variety of organisms that, besides shelter, also need a supply of water to conclude their life cycles [104]. In addition, the organisms living in the tank may provide important nutrients to the plant [101, 105].

Among Type III epiphytes it is possible to find C_3 and CAM photosynthesis (Table 1). In fact, some Type III bromeliads have been intensively studied in terms of photosynthetic plasticity. For instance, *Aechmea* 'Maya', which is a cross between *A. fasciata* and *A. tessmanii*, expresses CAM photosynthesis and has been used as a model for analyzing the impacts of several environmental factors (*e.g.*, light, nutrition, CO_2 availability) on the CAM behavior and carbohydrate partitioning [106-108]. Interestingly, when maintained under elevated concentrations of CO_2, this species increased the CO_2 uptake during Phases II and IV, but the nocturnal uptake of CO_2 remained similar to control conditions [106]. The extra CO_2 absorbed in those phases was used for the synthesis of hexoses that were not exported from the leaves. A later work on the same species showed that when these plants were transferred to low luminosity conditions, CAM was strongly dampened in the short term [107]. In fact, there is an acidification of the cytosol, which seems to be the result of an incapacity of the cells to degrade the malic acid formed during the previous night. The acid concentrations remained high for at least the first two days, resulting in serious damage to the cells. Also, CO_2 assimilation ceased and remained null at least until the sixth day of treatment. In the long term, the plant recovered a small part of the capacity to assimilate CO_2, when compared to the levels observed for control plants [107]. Accordingly, this species had a similar response when the four seasons were taken into account, with a higher level of carbon assimilation in more illuminated seasons (summer and spring) than in darker ones (winter and autumn - [108]).

Another Type III species that is receiving more and more attention in CAM studies is *Guzmania monostachia*. Maxwell and cols. [109] noted that plants of this species accumulated less acid when the photosynthetic active radiation decreased due to the season of the year or shading. Later, Maxwell and cols. [110] verified that high light and drought had a positive effect on CAM expression in this species, along with a powerful photoprotective mechanism, which

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could explain how *G. monostachia* couples with changing light and water supply in its environment. In fact, it was further described that when exposed to full sunlight, this species increases its accumulation of acids along with its photoprotective mechanisms within five days of the start of the dry season [111]. More recently, Freschi and cols. [90] studied the up-regulation of CAM in response to drought in *G. monostachia*. The authors found that the plants presented CAM-idling after seven days of drought. Another interesting feature brought forth by these authors is the differences in CAM expression along the length of the leaves, relating them to those observed in other bromeliads by Popp and cols. [83]. In the case of *G. monostachia*, there was an up-regulation of PEPC activity, followed by an increase in nocturnal acid accumulation only in the apical portion of the leaves. This observation agrees with other data regarding Type III bromeliads, showing that there is a division of functions along the leaf length (Figure 5). This is easily understandable, since the leaves of these species must perform both absorption of water/nutrients and photosynthesis. The base of the leaf, which receives less light and is in direct contact with tank contents, may be more specialized in absorption, while the apex, which intercepts more light, may be specialized in photosynthesis.

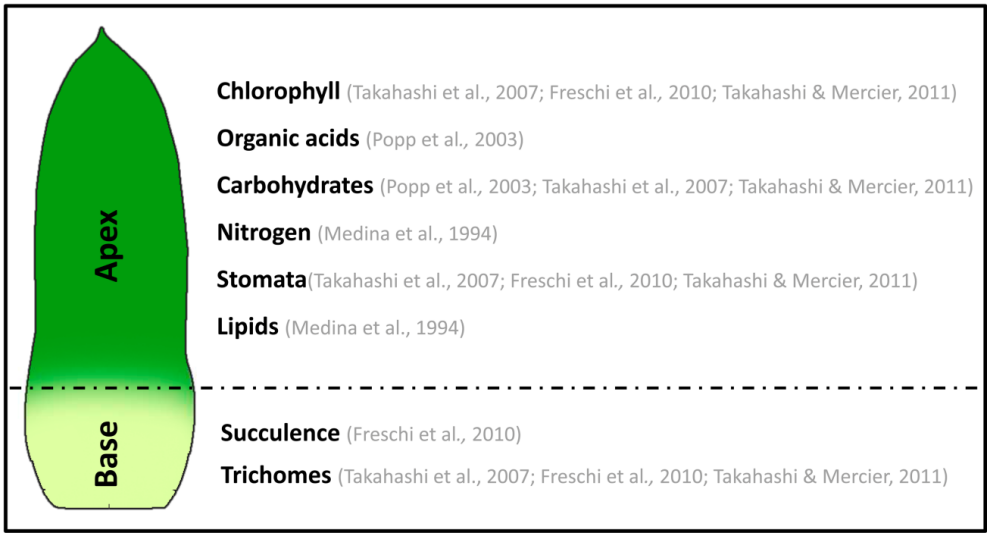

Figure 5. Comparative characteristics between apex and base of Type III bromeliad leaves. Characteristics described in the different leaf portions illustrate that they are more abundant in that region than the other.

However, some differences in gas exchange along the leaf were also observed, even if to a lesser extent, in other species that do not form the tank, like the Type I bromeliad *Ananas comosus* [112] and agaves such as *Agave sisalana* (James Hartwell, personal communication). This leads to the possibility that other factors besides functional specialization may be at work. In fact, both agaves and bromeliads have a similar structure: they are monocarpic monocots with the leaves disposed in a rosette (Figure 6). Therefore, these plants have a structure that always places younger leaves inside the rosette and at a greater angle than

those of the periphery [83]. Thus, light interception changes with leaf age [112]. Each individual leaf also has an age gradient along its length, due to the action of an intercalary meristem present in the leaf bases. Consequently, the apices of the leaves are older than their bases, and since CAM can be developmentally regulated, this would at least partially account for this base-apex gradient pattern. If this is true, then, this phenomenon should be found in all rosette monocots, regardless of whether they have a tank or not. It is probable, however, that the presence of the tank enhances the differences observed in the basal and apical portions of the leaf. The apparent occurrence of a base-apex CAM expression gradient along the leaves of the other non-bromeliad rosette plants, such as agaves, is discussed later in this chapter.

In Type III bromeliads, recycling of internal CO_2 seems to be an important resistance mechanism which allows the plant to keep stomata closed for a longer time and, therefore, save more water. In fact, Stiles and Martin [97] demonstrated that, when *Tillandsia utriculata* remained without water for several days, the stomatal conductance diminished, causing an increase in the proportion of refixed CO_2. This extreme response to drought can be even more important for Type IV bromeliads, which are presented next.

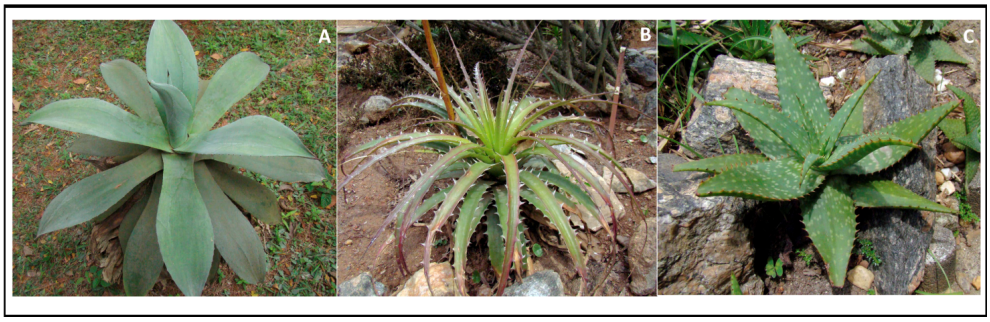

Figure 6. Monocot rosette plants. A. *Agave* sp. B. *Dyckia* sp. C. *Aloe* sp.

4.4. Type IV Bromeliads

Type IV bromeliads are the so-called atmospheric ones. In the absence of a tank, their leaves are covered by absorbing trichomes, which allow the uptake of water and nutrients that are suspended in the atmosphere or during rainfall [78]. The trichomes also seem to be useful in reflecting light to avoid photoinhibition [113, 114]. Therefore, atmospheric bromeliads must have a very efficient mechanism to capture water and nutrients as quickly as possible when these resources are available [102]. These species also need ways to save the absorbed water. In fact, all atmospheric *Tillandsia* species present CAM [68]. Martin and Adams III [95] measured the 24h gas exchange pattern in the CAM plant *Tillandsia schideana*, using it to estimate the amount of acid that should accumulate during the night, based on the amount of CO_2 fixed. When they measured nighttime acid accumulation, however, the values were much higher than the estimate. The authors concluded that this excess in acidity originated from

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recycling of the respired CO_2. Moreover, drought exposure caused a drop in CO_2 assimilation, and this difference between estimated and measured acidity values increased even more [95]. It is possible, then, that this species was on its way to CAM-idling as a resistance mechanism to withstand the drought period.

By comparing nighttime atmospheric CO_2 uptake and malate accumulation in 12 *Tillandsia* species, Loeschen and cols. [93] verified that respiratory CO_2 recycling significantly occurred only in *T. schideana*. One possibility is that these were well-hydrated greenhouse plants, so the rates of CO_2 recycling would be low when compared to CO_2 assimilated during night. In another study, Nowak and Martin [94] analyzed the CAM responses of *Tillandsia ionantha* under drought for 60 days. From the 30th day on, the CO_2 uptake started to drop until the 60th day. When comparing it to the acid accumulation, the authors observed that in response to drought the percentage of recycled CO_2 increased. Based on these data, it seems that *T. ionthana* was also on its way to a CAM-idling state in order to withstand drought. Later, Haslam and cols. [96] demonstrated that increasing irradiance was also capable of enhancing CO_2 recycling, along with water shortage in the atmospheric *T. usneoides*. A recent work with the atmospheric *T. pohliana* found that this plant accumulates almost equimolar concentrations of malate and citrate [91]. This is interesting because citrate accumulation does not result in a net carbon gain [115]; therefore, this accumulation may serve other purposes. Freschi and cols. [91] suggest that, since MDH is inhibited by OAA, in this species citrate formation would be a way to avoid excessive OAA accumulation. Moreover, citrate formation generates NADH, which could be consumed during the night by MDH and other enzymes. In fact, the same authors found that nitrate reductase, a great consumer of NAD(P)H, is mainly active at night in this species. Therefore, Type IV bromeliads are very good examples of plants that are capable of performing distinct CAM modes.

In conclusion, CAM can be found in Type I bromeliads when they inhabit xeromorphic areas, such as deserts, following a distribution similar to other plants, including other non-bromeliad terrestrial monocot rosette plants (*e.g.*, agaves, aloes). Type II would allow some accumulation of water, but since the contents of the tank are not so well absorbed and they have access to soil water, they seem to follow a similar CAM expression pattern to that found in Type I bromeliads. Among Types III and IV, the presence of a tank is perhaps a major difference that permits C_3, or at least facultative CAM, in the epiphytic habitat since water accumulation and absorption through this structure is possible. Without the tank, atmospheric bromeliads (Type IV) have to perform CAM or they will not survive. Therefore, the presence of the tank in some epiphytic bromeliads could be the reason why a clear correlation between CAM and epiphytism was not found in Bromeliaceae, different from other families with epiphytic species [4, 68, 80].

5. CAM in Agavaceae

When compared with the relatively abundant literature about CAM photosynthesis in bromeliads, much less is known about the photosynthetic biochemistry, regulation and

plasticity in Agavaceae. As many bromeliads, most Agavaceae representatives are terrestrial monocarpic rosette plants, inhabiting predominantly arid and semiarid regions. Similar to other xeric plants, many species of Agavaceae exhibit relatively low growth rates under both natural and optimal environmental conditions. On the other hand, sometimes impressive rates of biomass productivity can be found in *Agave* plants [116], which might be associated with their wide range of anatomical and physiological adaptations to survive and thrive under water-limited conditions. Among the physiological adaptations exhibited by Agavaceae representatives, the capacity to express CAM photosynthesis naturally deserves to be highlighted and, therefore, will be the main focus of this chapter section. The current and ancient uses of agaves for food, beverages and diverse natural products have already been recently covered by excellent reviews [116-118] and will not be emphasized in this chapter.

According to APG III in 2009 [77], Agavaceae belongs to the expanded family Asparagaceae, and *Agave* is currently treated as one of the 18 genera of the subfamily Agavoideae [119]. The genus *Agave sensu stricto* (166 species), which is divided into the two subgenera *Littaea* (53 species) and *Agave* (113 species), is principally monocarpic and covers the most succulent and dry-adapted members of the family. Among other adaptations, large succulent rosettes, which funnel water, and shallow root systems, which allow a rapid uptake of sudden precipitation, are traits commonly found among *Agave* species. Diversification and speciation of *Agave*, the largest genus of the family, were significantly elevated between 8-6 million years ago (late Miocene and early Pliocene), coinciding with an increase in dry conditions in central Mexico [120, 121], and then again between 3-2.5 million years ago (late Pliocene), coinciding with the distribution of nectarivorous bats, the main pollinators of current *Agave* species. The diversification of *Agaves* in North America during the phenomenon of reduced precipitation and atmospheric CO_2 availability in the late Miocene-Pliocene was simultaneous with the diversification of several other succulent plant lineages across the world, such as the ice plants in south Africa [122].

In contrast to observations in the Bromeliaceae, virtually all Agavaceae genera are believed to have at least some species capable of expressing CAM photosynthesis [123, 124]. Naturally, a certain number of Agavaceae representatives, such as some *Yucca* species, have been clearly demonstrated to be C_3 plants [125, 126]. For succulent agaves, CAM seems to be a ubiquitous trait, expressed many times in a quite rigid pattern with reduced gas exchange at Phases II and IV. For instance, it is known that species such as *Agave deserti, A. fourcroydes, A. tequilana, A. angustifolia, A. lecheguilla, A. lurida, A. murpheyi, A. parryi, A. salmiana, A. scabra, A. schottii, A. shawii, A. sisalana, A. utahensis, A. vilmoriniana, A. virginica* and *A. weberii* typically perform CAM photosynthesis under natural conditions [127-131]. Nevertheless, it was reported that *Agave deserti* is clearly able to change from CAM to C_3 photosynthesis when maintained under well-watered laboratory conditions [132]. In fact, a complete switch from CAM to C_3 diel gas exchange pattern with almost all net CO_2 uptake occurring during daytime and virtually no day/night acid fluctuations was observed in *A. deserti* plants maintained under well-watered greenhouse conditions, reinforcing the notion that a possible C_3-CAM facultative behaviour might occur in this particular agave [132]. Moreover, a certain level of photosynthetic plasticity has even been described for young and adult plants of the CAM constitutive *A. tequilana*, which

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allows them to modulate the contribution of daytime (Phases II and III) and nighttime (Phase I) carbon acquisition when facing different environmental conditions [133-134]. In fact, although most CO_2 uptake in *A. tequilana* takes place at night (Phase I) [133-135], the relative contribution of daytime carbon gain (especially in Phase IV) can be modulated throughout the year [134]. Curiously, in both adults and young individuals of this species, at least some Phase IV CO_2 acquisition can be maintained even during the driest months of the year, which is a phenomenon not very commonly observed among other CAM plants growing under arid conditions [133, 134].

In addition to optimizing carbon gain and growth, the occurrence of some daytime gas exchange might also contribute as a transpiration cooling mechanism, which would significantly benefit these plants as long as enough water is available in the soil or inside their succulent leaves and stems [136]. This daytime transpiration cooling system can help tropical plants minimize excessive increases in leaf temperature and is logically not present when stomatal opening is restricted to the night period (Phase I). Interestingly, in a recent study, it was verified that the spike (young folded leaves in the centre of the rosette) of *A. tequilana* is the most thermotolerant part of the plant, presenting the highest stomatal density and elevated levels of HSPs (heat-shock proteins) [136]. Considering the fact that the central spike is the youngest part of the agave, it seems plausible to suggest that these young tissues might exhibit lower levels of CAM expression and perhaps higher daytime gas exchange, which would inexorably lead to at least some transpiration cooling during the light period.

Whether young tissues of agaves present lower levels of CAM photosynthesis still remains to be investigated, but some current observations seem to indicate a possible influence of tissue age on CAM expression in these plants. For instance, as observed for rosette bromeliads [90], some base-apex longitudinal gradient in CAM expression might also be present along the leaves of *Agave sisalana*, in which CO_2 uptake in the more basal and younger leaf portion occurred almost exclusively during daytime, whereas a gas exchange pattern typical of CAM photosynthesis was observed in the more mature leaf tip (J. Hartwell, personal communication). In this study, conducted in detached leaves, all leaf portions received the same amount of PAR incidence, ensuring that these changes in CO_2 uptake pattern would not simply be a result of distinct light availability along the leaf blade. Further suggesting some influence of plant ontogeny on CAM photosynthesis in Agavaceae species, Olivares and Medina [112] observed that nocturnal changes in titratable acidity were also dependent on leaf age in *Fourcroya humboldiana* since this parameter increased with the distance from the leaf bases and also from the younger to more mature leaves, reaching maximum values at the 7^{th} leaf (counting from the rosette centre). Moreover, it has also been demonstrated that late-afternoon CO_2 uptake (Phase IV) in *A. deserti* decreases as the seedlings age, being virtually absent in adult plants of this species [137], which might indicate a transition to a CAM mode more strictly dependent on nighttime (Phase I) CO_2 uptake. As in other CAM plants, factors like leaf arrangement, total daytime PAR, daytime/nighttime temperature and drought seems to influence CO_2 uptake in *Agave* plants [129, 135, 138]. For instance, it has been suggested that *Agave* required more than 4 mol photons m^{-2} d^{-1} (93 μmol m^{-2} s^{-1} for 12-photoperiod) to fix CO_2 at night and, therefore, to accumulate organic acids [129]. In *A. fourcroydes*, nearly 90% of

PAR saturation of nocturnal CO_2 uptake happened near a total daily PAR of 20 mol m^{-2} [138], values comparable to other CAM plants. Also in this *Agave* species, studies have demonstrated that when drought conditions were imposed, a higher fraction of daytime CO_2 uptake was lost compared to nighttime CO_2 uptake [138]. In fact, adult *A. fourcroydes* plants exposed to 11 days of drought exhibited a reduction of 99% in net daytime CO_2 uptake and 76% in nighttime CO_2 uptake (Nobel, 1985b). In addition, it has been demonstrated that low leaf temperatures at night are quite beneficial for nocturnal gas exchange and organic acid accumulation in different *Agave* species [127, 135, 138]. Based on the lack of information about CAM in Agavaceae, additional studies are required to determinate whether CAM photosynthesis in *Agave* plants really depends on the plant and/or leaf ontogenetic stage and whether it can be facultatively expressed in some species in response to environmental stimuli. Naturally, the regulatory mechanisms controlling CAM expression in these plants remains even more elusive.

As commonly observed in many other CAM xerophytes, leaf succulence is a widespread feature of agaves. The internal water storage provided by a prominent leaf hydrenchyma might be, at least in part, responsible for the relatively high biomass productivity observed in some *Agave* species living in severely water-limited habitats. This prominent succulence would serve to buffer abrupt and long-term changes in water availability, helping to maximize nocturnal CO_2 uptake and extend the duration of atmospheric CO_2 acquisition beyond the night period [134]. As in other plants displaying the "succulent syndrome", the presence of CAM photosynthesis and leaf succulence in agaves is also correlated to features that reduce water loss, like thick cuticles, reduced stomatal size and/or frequency, and other water-conserving characteristics. As a result, remarkably high water use efficiency (WUE) is usually observed in *Agave*, allowing these plants to colonize dry heterogeneous environments, sometimes even achieving elevated productivity values at these locations. Naturally, precise adjustments in photosynthetic biochemistry are clearly needed to obtain the highest day- and nighttime CO_2 uptake possible with the usually scant and erratic water supply of arid and semi-arid regions.

Studies have demonstrated that the prominent leaf succulence of several adult *Agave* species is key for allowing the occurrence of substantial net CO_2 uptake even when soil water content reaches relatively low levels, reinforcing the importance of this internal water supply to ensure high photosynthetic performance during the entire year [133]. Interestingly, though, young plants of *Agave tequilana*, even while presenting lower succulence than adult individuals and, therefore, comparatively lower internal water storage, were able to obtain carbon during day and night under field conditions. These plants exhibited almost the same carbon gain of adult individuals and maintained relatively high photosynthetic assimilation rates during both dry and wet seasons [134]. Naturally, the continuous water movement from the medullar hydrenchyma to the marginal chlorenchyma during the dry season, when soil water availability can decrease to critical values, might in fact be a critical factor for allowing the occurrence of relatively high levels of CO_2 assimilation year-round, even in these young agave plants [134]. Under the severely dry conditions normally faced by many agaves, internal water storage tissues such as hydrenchyma are obviously much more appropriate than an external water

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reservoir such as the tank of certain bromeliads. Besides the scarce and sporadic rain events, the air humidity of arid and semi-arid regions would inexorably lead to a fast evaporation of any external water reservoir, whereas internal water storage would last much longer by benefiting from additional morphological, anatomical and physiological adaptations, such as thick cuticles, highly regulated stomata control, accumulation of osmotically active compounds in the water storage tissues, among others.

In this sense, another typical feature of Agavaceae species is the production of fructans, which are polymers of β-fructofuranosyl residues synthesized from sucrose and accumulated in the vacuoles of succulent parenchymatic cells of leaves and stems. Fructans are believed to contribute in several ways to plant metabolism and development, including osmoregulation, cryoprotection, and drought tolerance [139-141], and in mature agave plants fructans become an energy source for flowering. In general, the type and structure of fructans can be indicative of the species, within the limits of effects triggered by the environment and growing stage of the plant [116, 142]. Being water soluble and, therefore, osmotically active, fructans can influence the osmotic potential of parenchymatic cells. This osmotic impact of fructans might depend, among other factors, on their degree of polymerization and relative concentration inside the vacuoles of each cell. Since fructans are not as highly polymerized as glucans (*e.g.*, starch), their use as main storage carbohydrate might be of significance for determining the osmotic pressure in agave cells [112]. Although some evidence suggests that fructans are not broken down during the dark period to provide PEP as substrate for the nocturnal CO_2 fixation in agaves [143], other data indicate a possible use of these carbohydrates as the main source of nighttime PEP production [112]. For instance, it has been observed that diel fluctuations in sucrose could account for more than 83% of the carbon needed for nocturnal PEP regeneration in *Agave americana* [143]. On the other hand, in *Agave guadalajarana* diel fluctuation in the leaf starch, glucose, fructose and sucrose could not account for the carbon required for nighttime PEP production, and a possible use of alternative extrachloroplastic carbohydrates (such as fructans) for PEP generation has been suggested [144]. This suggestion is in agreement with the relatively low content of starch normally observed in *Agave* tissues and with the inverse relationship between fructans and malic acid observed in some Agavaceae species such as *Fourcroya humboldiana* [112]. In fact, the leaf levels of soluble fructans (including sucrose and fructose) in *F. humboldiana* clearly decreased during the night coinciding with the period of malic acid accumulation. The amount of carbon involved in these reciprocating fluxes indicated that fructans apparently represent the exclusive source of PEP for dark CO_2 fixation in this Agavaceae species [112]. Diel changes in the degree of polymerization of *F. humboldiana* fructans were also observed, which might be associated with the hydrolysis of fructose molecules from the fructans during the night for PEP regeneration and a reverse process during the day when CO_2 from malate would be incorporated into new fructose units of the pre-existing fructan molecules [112].

In summary, the impacts of environment and development on CAM expression capacity and carbohydrate metabolism in *Agave* species are still poorly understood. This extensive lack of knowledge regarding the relevant traits that account for the capacity of these plants to productively grow under arid and semiarid conditions contrasts diametrically with the

enormous economical potential that some *Agave* species possess for biomass and renewable material production in marginal lands. This situation might possibly change in a near future, especially considering the increasing interest in using these and other CAM species as alternative crops in a context of global climate change and increased desertification.

6. Bromeliads and agaves CAM plants in a climate change and desertification context

Climate change involves elevated CO_2 concentrations, increasing temperatures and/or changes in precipitation patterns. Therefore, the perspective of climate changes around the planet has stimulated extensive research into assessing the impacts of elevated CO_2, elevated temperature and drought on different vegetation types. The atmospheric CO_2 concentration has been increasing rapidly during the last century, now reaching about 390 ppm. Promoted by deforestation, land-use, and the burning of fossil fuels, CO_2 is predicted to double by the middle of this century. Besides being an important change in the environmental conditions in and of itself, this progressive increase in atmospheric CO_2 concentration might indirectly impact climate by leading to increases in global temperature and perturbations in precipitation patterns.

The effects of elevated atmospheric CO_2 on carbon gain, plant growth and physiological performance depends on the CO_2 assimilation pathway, the exposure duration and the environmental conditions, among other factors [106]. For instance, growth under CO_2 enrichment might impact the relative contribution of C_3 and C_4 carboxylation pathways to net carbon gain, which could affect WUE over the day/night cycle and carbohydrate fractioning for growth and export [106]. As CAM plants use Rubisco and PEPC to take up CO_2, different conclusions have been reached. For instance, it has been proposed that PEPC in CAM plants might be saturated at the current atmospheric CO_2 concentration [145, 146]. However, divergent results about the influence of elevated CO_2 in CAM plants have been reported during the last decades [147-153], demonstrating that the enrichment of CO_2 into the atmosphere can trigger complex responses in CAM plants. For example, elevated CO_2 had no effect on diel CO_2 uptake by *Kalanchoë daigremontiana* [147] nor on nighttime CO_2 uptake in *Clusia uvitana* and *Portulacaria afra* [154, 155], whereas in several other CAM species more significant impacts of elevated CO_2 on the daytime, nighttime and/or diel carbon acquisition have been reported [148, 150, 151, 153]. Changes in morphology, anatomy and biochemistry driven by modifications in atmospheric CO_2 concentration have also been observed in some CAM plants, commonly associated with concomitant alterations in their growth rates and biomass accumulation. Moreover, in plants maintained under elevated atmospheric CO_2, some researchers have observed instantaneous net CO_2 uptake increasing over time, which suggests that the response to high levels of CO_2 in the atmosphere is maximized by physiological and morphological changes, such as chlorenchyma thickening [149, 152, 156]. Since leaf succulence limits diffusion of CO_2 and optimizes the accumulation photosynthetic products, changes in this morphological trait over time might contribute to hamper the acclimation of CAM plants under elevated CO_2 in the atmosphere.

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Among bromeliads, perhaps the first study with the goal of evaluating the influence of elevated CO_2 on CAM photosynthesis was carried out by Nowak and Martin [157] in the atmospheric epiphyte *Tillandsia ionantha*. In this study, the authors demonstrated for the first time that a CAM plant could respond to high atmospheric CO_2 with significant increases in nocturnal malate accumulation, which would potentially lead to increases in productivity. Subsequent studies conducted with pineapple (*Ananas comosus*) plants have demonstrated that under elevated CO_2 concentration, this species responds with increases in both morning and nighttime CO_2 uptake [150, 151], associated with higher values of WUE [151], productivity, root:shoot ratio and leaf thickness [149]. Interestingly, when the pineapple plants were heavily irrigated, CAM activity and biomass response to elevated CO_2 were significantly reduced [151, 158].

The responses of bromeliads of the genus *Aechmea* have also been investigated under elevated atmospheric CO_2 concentrations [106, 158, 159]. Long-term exposure of *Aechmea magdalenae* to double CO_2 concentration (~ 700ppm) resulted in improved growth, although non-significant increases in daytime and dark CO_2 fixation were observed [158]. For *Aechmea* 'Maya' it was evidenced that an atmosphere of 700 ppm CO_2 promoted a 60% increase in carbon gain, through promotion of diurnal C_3 pathway and C_4 carboxylation during Phases II and IV, where WUE was equivalent to night periods [106]. Elevated CO_2 promoted the accumulation of hexose during Phase IV, stopping neither net daytime carbohydrates export nor the stimulation of dark carboxylation and nocturnal export [106]. These authors point out respiration as the major carbohydrate sink in *A. Maya* and recognized discrete pools of carbohydrates for CAM and for export. They observed a two-fold increase in water use efficiency under elevated CO_2, suggesting this as the major physiological advantage of CO_2 enriched atmosphere, which can be favourable for growth during drought stress events [106]. Curiously, studies conducted on *Aechmea* plants with ornamental value, like *Aechmea* 'Maya' and *Aechmea fasciata* 'Primera', showed different responses under elevated CO_2 atmosphere [159]. For example, during 34 weeks of growth under CO_2 enriched atmosphere, *A.* 'Maya' biomass and leaf micromorphology showed no significant changes, whereas elevated CO_2 promoted a reduction of total leaf area and thickness in *A. fasciata* 'Primera', which led to a reduction in fresh and dry biomass. Curiously, during these changes driven by elevated CO_2, some ornamental traits were lost in *A. fasciata* 'Primera', especially due to the reduction in total chlorophyll and the changes in leaf allometric length/width ratios, producing paleness and more compact plants [159].

Thus far, studies on the impacts of elevated CO_2 on Agavaceae species have been mainly conducted in the genus *Agave* and *Yucca* [126, 156, 160-162]. *Agave deserti*, *A. salmiana* and *A. vilmoriniana* plants grown under elevated CO_2 concentrations showed higher nighttime and/or afternoon CO_2 uptake [156, 160-162] as well as increased WUE and productivity [156]. In *A. deserti*, elevated CO_2 treatment for 17 months resulted in longer and thicker leaves, thicker chlorenchyma and increased root cell length [156]. Moreover, in *A. deserti* and *A. salmiana*, long-term treatments with elevated CO_2 resulted in decreases in total PEPC and Rubisco activity associated with increases in Rubisco *in vivo* activation status [156, 160], which can be interpreted as a strategy for maintaining photosynthetic performance since increases in the activated *vs.* total ratio for Rubisco would compensate for decreases in total activity of this

enzyme. Also in *A. deserti* and *A. salmiana*, increases of up to 30% in dry weight gain have been reported after long-term treatments under elevated CO_2 [161]. On the other hand, significant differences in dry weight accumulation in *A. vilmoriniana* grown for 6 months under 350 or 675 ppm of CO_2 were only observed when these plants received water just once a week, but not when they were watered twice a week [163].

Taken together, these reports, sometimes presenting contrasting results, may reflect the inherent plasticity of CAM in terms of optimizing carbon gain and WUE in a changing scenario [106]. The impressive absence of acclimatization in CAM plants to elevated CO_2 enrichment can be perhaps correlated with the prominent succulence observed in many of these species, which might proportionate a large space for accumulating photosynthates without feedback inhibition [152]. Naturally, more research about carbohydrate partitioning in CAM species is clearly needed for a better understanding of the lack of acclimatization of these plants under elevated CO_2. It is also important to keep in mind that all the conclusions currently available about the impacts of high atmospheric CO_2 on CAM photosynthesis are still based in relatively few studies and species.

Since most predictions about the future global climate scenario describe intensification in aridity, with possibly increased desertification around the world, the adaptive success of CAM plants in these challenging environments might represent an important alternative for carbon sequestration and use as crops for production of biofuels and other renewable materials in regions not suitable for cultivation of agronomic species performing C_3 and C_4 photosynthesis [11, 49]. The capacity of CAM plants to survive and productively grow in environments subjected to frequent and/or long-lasting dry periods, especially due to their ability to improve net CO_2 assimilation and WUE by carefully adjusting the period for capturing CO_2 during the 24-hour daily cycle, is certainly a feature that justifies even more intensive research on these plants in both the short and long term. Considering the fact that some CAM agaves (*e.g., Agave salmiana, A. mapisaga, A. tequilana, A. americana, A. sisalana*) and bromeliads (*e.g., Ananas comosus*) can achieve productivity levels only slightly lower than those found in C_3 and C_4 species [164], these plants might represent special targets for studies aimed to optimize their use both at the current and future global climate scenarios.

7. Conclusions

It has been hypothesized that the evolution of CAM in terrestrial and aquatic environment was favored by decreasing atmospheric CO_2 levels and CO_2/O_2 ratio during the Miocene. Thus, atmospheric CO_2 reductions, coupled with warm climates in subtropical latitudes, were the most important driving forces in the evolution of CAM. Then, CAM photosynthesis may have originated as a means of improving carbon economy. Phylogenetic reconstructions from comparative physiology and taxonomy have shown that CAM appeared multiple times and originated from a C_3 ancestor since all enzymes related to CAM functions are also found in C_3 plants. These facts suggest that metabolic pathways related to CAM have arisen from co-option processes.

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Remarkable differences between C_3 and CAM metabolisms continue to intrigue many researchers, and the importance of many of these differences on CAM functioning is still not completely understood, for example, the importance of the contribution of C_4-carboxylation or C_3-carboxylation in reproductive development of CAM plants; in addition, nowadays very few explanations about the existence of different starch breakdown pathways in C_3 and CAM plants are currently available. It is possible that Glu-6-P exportation at night from the chloroplast (phosphorolytic pathway) in CAM plants could be related with the allosteric activation of PEPC, but this issue has not been directly studied yet.

Nowadays, it is possible to observe that Crassulacean acid metabolism is a remarkable adaptation to environments with low abundance of water, showing a great plasticity in its expression. This plasticity resulted in a classification of several types of CAM (*e.g.*, CAM cycling, CAM idling, *etc.*), and it is important to keep in mind that even constitutive CAM plants do not appear to perform one of these types exclusively, instead transiting between them in response to environmental or developmental conditions. C_3-CAM plants show an even greater plasticity, being capable of going from the C_3 mode to CAM mode, also as a result of developmental or environmental cues. Nevertheless, it is currently almost impossible to establish clear differences between facultative CAM and constitutive CAM plants since it has already been demonstrated that even some constitutive CAM plants, when juvenile, are capable of switching from C_3 to CAM and return to C_3 mode, in a fully reversible way. Therefore, due to the facultative component that exists in constitutive CAM, we propose that facultative CAM species should be those capable of going from C_3 to CAM and back even in adulthood and not just during the juvenile stage. On the other hand, species capable of irreversibly expressing CAM at some moment in their life cycle, regardless of whether CAM expression is influenced by either environmental or developmental factors, should simply be considered as CAM plants.

Bromeliaceae is a very important neotropical plant family with several CAM-performing species. This family has a variety of life forms, which allows the individuals to cope with different kinds of environments, from semi-arid to rainforests, terrestrial to epiphytic habitats. As a water saving mechanism, CAM is undeniably linked to environments in which water is limited for some reason, including semi-arid regions or the epiphytic niche. Thus, a higher abundance of CAM plants is expected to be found in these niches. This is true for semi-arid environments, yet in the epiphytic habitat this observation is controversial: CAM does not seem to be linked with epiphytism in Bromeliaceae. The response to this apparent contradiction could depend on how each species minimizes the lack of water in the environment. For example, the presence of the tank could provide water for the plant in a longer term, thereby allowing tank bromeliads to be C_3-CAM plants or even exclusively C_3, when almost all epiphytes without the tank could be CAM-performing species.

In tank bromeliads, another issue that draws attention is the division of functions inside a single leaf. The basal portion of the leaf seems to be responsible mainly for absorption of water and nutrients, while the actual photosynthetic activity is more restricted to the apex. This difference of functions could be, at least in part, the result of an intercalary meristem, present in monocot leaves. The activity of this meristem generates a gradient of juvenility along the

leaf, the apex being the oldest portion. This may be one of the reasons why other species with an architecture similar to that of bromeliads (like agaves, for example) also show some differences in photosynthetic behavior along their leaves.

Besides sharing similarities with bromeliads in terms of general architecture and photosynthetic activity along the leaves, Agavaceae species also seem to present some degree of flexibility in CAM expression. For instance, the ability to switch from C_3 to CAM metabolism in response to environmental cues has already been suggested for at least one agave, *A. deserti*, and more research is needed to clarify whether CAM expression in other species of Agavaceae family would also be controlled by environmental cues and/or ontogenetic program. On the other hand, while many bromeliads usually rely on external water reservoir (*i.e.*, tank) for coping with periods of water shortage, agaves normally display large internal water storage (*i.e.*, medular hydrenchyma) to survive during the harsh xeric conditions where they usually inhabit. In addition, particular water soluble carbohydrates (*i.e.*, fructans) are usually stored in the medullar hydrenchyma of agaves, possibly helping these plants to osmotically adjust the pressure in their cells under variable conditions of water supply.

Finally, the impacts of elevated CO_2 on CAM bromeliads and agaves seems to be relatively variable, possibly indicating that these plastic plants might display distinct strategies to adapt their photosynthetic activity to changes in this environmental factor. The combinatory effects of high CO_2 and other environmental changes predicted for future global climate scenarios (e.g., high temperatures, intensified aridity, etc.) on the physiology of these plants still need to be better defined through more extensive studies involving a wider range of bromeliad and agave species. The possible economical use of these and other CAM plants as alternative crops in a future scenario of increased temperature and aridity is currently a topic of great interest in the research community.

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