

The effects of environmental factors in the induction of Crassulacean Acid Metabolism (CAM) expression in facultative CAM plants

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Abstract

In extreme habitats, plants exhibit versatile photosynthetic pathways in response to environmental variables such as temperature and moisture. One such variation of carbon acquisition, Crassulacean Acid Metabolism (CAM), is often employed by plants in arid regions, where they usually face a “desiccation-starvation dilemma” because they have to acquire enough carbon to survive while minimizing the amount of water loss through stomatal activities and other physiological and biochemical functions. CAM photosynthesis allows increased water conservation and CO₂ concentration because the stomata open at night when the transpiration rate is low; therefore, CAM photosynthesis is adaptive in arid regions. Some CAM plants are described as “facultative” CAM plants because they are highly flexible in their mode of photosynthesis and can switch to C₃ photosynthesis (i.e. stomata open during the day) when water is abundant. This review will focus on the ecological and physiological significance of this trait by discussing the environmental exposures necessary to trigger facultative CAM and their correlation between the plants and environmental factors such as CO₂ level, water, salinity, temperature, nutrient and light. Discussion on how plants use facultative CAM to avoid stress and how their physiology contributes to this process will also be addressed. Although CAM is fundamentally a biochemical pathway, this review will mainly focus on the interaction of ecological conditions and physiological characteristics of plants that employ facultative CAM.

Introduction

Crassulacean Acid Metabolism (CAM) is a modified photosynthetic adaptation that temporally separates the C₄ pathway and the Calvin Cycle, and plants that utilize this type of photosynthesis fix CO₂ during nighttime. The stomata close during the day, retarding water loss, and open during the night. This action results in a low transpiration rate and high water-use efficiency. CAM photosynthesis is adapted by a large number of vascular plants in arid regions for water conservation and CO₂ concentration. The nocturnal CO₂ fixation is done by the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPC). The CO₂ is fixed into malate, and stored in the vacuole within the same cell as malic acid- an organic acid that serves as a CO₂ reservoir for daytime CO₂ re-assimilation by the normal C₃ (Calvin Cycle) photosynthetic pathway [1, 2].

CAM plants exhibit a wide degree of plasticity in their expression of the CAM pathway. For instance, facultative CAM plants are highly flexible in their method of carbon acquisition in that they utilize the C₃ pathway during favorable conditions such as mild temperature and abundant precipitation, but switch to a CAM mode when stressed (e.g. drought) [2-4]. The level of expression from C₃ to CAM in plants is on a continuum, with C₃ and CAM being the extremes. Once CAM is induced, it is highly reversible depending on various changing environmental conditions [5]. The C₃-CAM switch is important in maintaining viable physiological performance under stress; thus, facultative CAM plants are very sensitive to changes in environmental factors [1]. CAM plants occupy a wide range of habitats, and CAM induction in these plants is governed by a combination of environmental factors, severity of the stress, physiological factors, the seasonality, age and genotype of the plant. This review, however, will focus on the environmental

factors that induce CAM mode in facultative CAM plants.

The transition from C₃ to CAM is commonly induced by drought and soil salinity, but other primary environmental parameters that impact photosynthetic functioning are also involved (e.g. photoperiod, day/night temperature change) [6]. CO₂ level and water availability were likely the most prominent parameters as the initial driving forces of CAM evolution, and these parameters continue to influence CAM functioning significantly [2]. CO₂ levels have a more profound selection pressure on freshwater than land plants. The presence of algae in freshwater creates competition for CO₂ during the day, but CO₂ is more available through respiration during the night. Thus, practicing nocturnal carbon fixation, CAM usage may be advantageous for freshwater plants [1]. Water availability imposes an immediate stress on land plants, particularly those living in arid regions [2, 7]. However, water stress or CO₂ alone does not fully explain the wide range of CAM adaptations. Other important factors, including salinity, light, temperature and nutrients, determine CAM modes as well, and these factors may or may not be directly linked to water stress [2] (Supp. Fig.1). All these parameters will be discussed individually in this review. This review is significant in showing a detailed example of how phenotypic plasticity in plants relates to the environment, particularly as CAM plants are unique adaptations to some of the harshest habitats throughout the world.

Habitat

CAM plants are found in contrasting sites in many different ecosystems and are highly polyphyletic with approximately 16000 species [1]. Desert succulents account for 1800 species, and there are about 10700 species of rainforest epiphytes [2]. Facultative

CAM plants commonly exist in places with seasonal climate fluctuations, and unpredictable periods of stress [1].

Mesembryanthemum crystallinum is an annual plant frequently used as a model species for facultative CAM research [1]. The habitat of *M. crystallinum* along the coast of California, for example, is characterized by cold, wet winters, during which the plants perform C₃ photosynthesis. Then during the hot, dry summers with increased salinity, it employs almost exclusively the CAM mode [1, 3, 8]. Moreover, a facultative CAM species in the genus *Kitchingia* in Madagascar appears in forest climate zones during hot periods [2]. A species of *Kalanchoë* from Madagascar showed weak CAM in humid spots but strong CAM mode on bare rocks, suggesting that the CAM mode is related to micro-habitat structures [9]. Different lighting and flooding regimes also dictate the occurrence of CAM/C₃ populations [10].

Facultative CAM distribution is also limited by the temperature difference associated with latitudinal and altitudinal changes. Increasing altitude is generally associated with declining number of facultative CAM species and they disappear at 1175m [11]. Instead, obligate CAM is often employed at higher altitudes [2].

Physiology

Nocturnal CAM fixes CO₂ into malate, and large vacuoles are required to store this organic acid. Photosynthetic tissue cells are often densely packed to enhance transport capacity and to prevent daytime CO₂ leaching. These traits together create the succulence in CAM tissue anatomy [12]. Tissue succulence featured many independent convergent evolution events in the evolutionary history of CAM in various different plant families, such as spurge, cacti and milkweed [12]; succulence is the most prominent physiological characteristic of CAM that is considered essential for CAM biochemistry [2].

Succulence, however, is not the definitive physiognomy of CAM plants. From the 28 CAM species studied by Martin et al. [13], all facultative CAM plants were succulent, but overall, they observed no correlation between succulence and CAM. This suggested that succulence in a plant alone does not necessarily suggest a functioning CAM mechanism. Many epiphytic CAM plants, such as the Spanish moss, are not succulent. Some, such as members of the genus *Yucca*, can grow to tree size with secondary thickening [14]. *Clusia minor* is an example of a true dicotyledonous tree that exhibits facultative CAM [2].

Induction of CAM and its strength can be measured by nocturnal malate accumulation, titratable acidity, water content of the leaves, and the relative ratio of C₃-like and C₄-like carbon isotopes [15, 16]. Intermediate carbon isotope values can be observed in facultative CAM species and is a polymorphic character of CAM [17]. Stomatal activity is another useful indication because facultative CAM plants follow CAM pattern after induction (i.e. nocturnal stomata opening and diurnal closure) [1].

M. crystallinum has a special water storage feature of epidermal bladders that cover the plant surface [8]. When *M. crystallinum* develops in saline conditions, the new leaves are smaller and the epidermal bladders much larger [18]. Stress often increases the rate of CAM induction, but in *M. crystallinum*, CAM mode is also associated with maturation of the plant; young plants cannot express CAM [8].

Environmental Parameters

CO₂ Level

While CO₂ level may be considered the most important driving force in the evolution of CAM, CO₂ level seems to play a minor role in the induction of CAM in facultative CAM species. Under three CO₂ treatments of different concentrations, CAM mode was only induced in water-stressed plants but not well-watered ones [19]. The uptake of CO₂ by facultative species can be directly influenced by water status of their substrate, as water largely controls stomata activity. This is illustrated in *Sedum telephium*, a temperate succulent facultative CAM species, where CO₂ uptake was shown to differ under different water availability — well-watered plants fixed CO₂ at a rate of 2.2 μmolm⁻²s⁻¹ during the day, while drought plants only fixed CO₂ during the night, at a maximum rate of 0.69 μmolm⁻²s⁻¹ [5].

Water

The CAM pathway has high water use efficiency (WUE) due to the nocturnal stomata opening that leads to lower water loss via transpiration than plants with diurnal opening of the stomata. This feature has high ecological importance in terrestrial systems, especially in arid areas [1, 4].

CAM plants have additional mechanisms to deal with the limited water availability in certain environments. For instance, *M. crystallinum* illustrates how long-term water storage works in CAM with the large epidermal bladders over mesophyll tissues [8]. A water potential gradient drives water transport from the bladders into the mesophyll cells during midday, providing evidence of this important structural functioning at stressful times [20]. Additionally, roots of *M. crystallinum* are able to detect low soil water status and then transport signals to the leaves where the CAM pathway can be triggered [21]. This information is suggested to be conveyed by plant growth hormones abscisic acid (ABA) and cytokinin from the root to the leaves by transpiration [22].

Growth chamber experiments on *S. telephium* showed the complete C₃-CAM transition occurring in 8 days [5]. The respiratory CO₂ cycling went from 20% at day 1 to 44% at day 10; however, the CO₂ uptake capacity was reduced overall after 10 days of drought as the stress appeared to be too severe for the whole plant functioning [5]. In contrast, well-watered *S. telephium* performed C₃ photosynthesis exclusively. In an experiment by Brulfert et al. [23], *Kalanchoë minita* and *Kalanchoë porphyrocalyx* were subjected to 16 days of drought and CAM traits were emerging within 2 days of treatment. The two species performed quite differently at the end of the treatment: *K. porphyrocalyx* demonstrated a much higher drought resistance and *K. minita* had gone into a stage of using only recycled respiratory CO₂ (CAM-idling) [23].

The part of the plant being deprived of water is also important in determining CAM induction. Partial deprivation of root water content is enough to trigger CAM, without changing the leaf water content [21].

Salinity

Salinity creates osmotic stress and is strongly related to water status because water determines the concentration of any given substrate. CAM plants are generally highly salt-sensitive and practice strategies such as salt exclusion. The epiphytic CAM plants are typically salt-avoiders [2].

One exception is *M. crystallinum*, which can tolerate high salinity due to the ability to withstand high concentration of salt in their system [24, 25]. *M. crystallinum* appeared to be capable of retaining up to 400mM Na⁺ when treated in a 250 mM NaCl medium, and was able to keep tissue concentration constant in a 400mM NaCl solution [24]. *M. crystallinum* can even successfully reproduce in a 500mM NaCl medium [8]. CAM induction and the intensity of the CAM response in *M. crystallinum* is influenced by age and developmental factors respectively [3]. When treated with different concentrations of saline solution, high salinity treatments quickly induced CAM mode in young *M. crystallinum* and strongly accelerated the age-dependent process, whereas the lower salinity treatments performed C₃ photosynthesis exclusively [8]. In contrast, *M. crystallinum* grown in well-watered, non-saline treatments were able to complete their life cycles without ever exhibiting CAM [3].

Removal from the saline environment should reduce CAM expression. However, older leaves show lower reversibility, as shown by Ratajczak et al. [26], whose experiment demonstrated that at the same age, plants that were stressed would still maintain partial CAM expression after removal of stressor, whereas the non-stressed plants do not retain CAM mode.

Temperature

Air humidity is strongly tied to temperature- usually the higher the temperature, the drier it gets. Hence, temperature is involved in stomata regulation and CAM induction in facultative CAM plants [1]. *Clusia minor*, a facultative CAM tree species, has stronger CAM induction when diurnal temperature difference increases. In contrast, if temperature stays constant then the CAM response appears to be less sensitive [27]. This insensitivity is only associated with temperate species as many tropical species can have full CAM expression under constant temperature [2]. This provides evidence that CAM induction is induced by multiple factors.

Nutrient

In comparison to C₃ plants, CAM species use less nitrogen (N) because less Rubisco is required due to the CO₂ concentrating system [2]. Nevertheless, nutrient deficiency is problematic for any organism, and in many CAM epiphyte habitats, substrates are severely depleted of mineral nutrients.

In the facultative CAM species *Kalanchoë lateritia*, nitrogen concentration appeared to play a role as CAM was best induced at intermediate N level (NO₃⁻ = 2.894 mol m⁻³ and NH₄⁺ = 0.208 mol m⁻³) [28]. Ota [29, 30] found the chemical forms of nitrogen had different induction effects as well: for *Kalanchoë blossfeldiana*, an overall N-deficiency induced CAM, but ammonium ion (NH₄⁺) appeared to have weaker induction strength than nitrate (NO₃⁻). Induction of CAM in *M. crystallinum* is also affected by nitrate and phosphate deficiency [31].

Light

For any photosynthetic organisms, light is the direct source energy for photosynthesis and carbon acquisition. Light also controls the circadian cycle of the organism by acting as an important signaling system that activates many physiological functions including the induction of CAM expression in facultative CAM species [2]. The color, intensity and length of photo-periods all

contribute to CAM induction [32, 33].

Guard cell response is regulated by a blue-light photoreceptor in the C₃ photosynthesis mode [33]. Upon CAM induction, stomatal activity changes and the light response is then altered. In *Portulacaria afra*, a facultative species, C₃ and CAM individuals have dramatically different responses to blue and red light. The responses that are stimulated in C₃ mode appear to be lost or inhibited in the CAM mode [33]. In *M. crystallinum*, the opening of guard cells in response to blue light occurs in the C₃ mode but this response is lost after CAM induction [34].

In some facultative CAM species, CAM expression may be elicited by high light intensity [2]. In a tropical savannah site, Herzog et al. [35] studied *Clusia multiflora* (an obligate C₃ species) and *Clusia minor* (a facultative CAM species) and found that *C. multiflora* suffered from light damage whereas *C. minor* was able to avoid detrimental photoinhibition by shifting into CAM mode. The tropical savannah is highly variable because it is a transition zone between grassland and desert, and is characterized by extended dry seasons and brief wet summers. The *Clusia* example thus illustrated the advantage of C₃-CAM shift in environment with high amount of variability.

The length of the photoperiod is also important; brief exposure to high-intensity light may not be sufficient to generate the switch [2]. Typically, facultative species require day-long exposure to initiate CAM mode. This was observed in *M. crystallinum* and *K. blossfeldiana* [36].

Conclusion

It was suggested by Winter and Holtum [3] that CAM induction is controlled largely by environmental factors, rather than a pre-programmed genetic process. Nevertheless, upon external triggers, changes in the biochemical reactions within the plants, such as the formation of necessary proteins and the alteration in electron transport chain are what complete the CAM expression. Minor environmental factors, such as atmospheric input of sulphite, a widespread air pollutant [37], and the developmental stage of the plant are also important contributing factors to consider [4]. It is also important to keep in mind that, despite the sufficient water conserving mechanism that allows CAM plants to do well in extreme aridity, they are poor competitors to C₃ and C₄ plants in normal temperature or moisture regimes. This fact shows that it may not be forever advantageous to utilize the CAM pathway. Under the possible influence of climate and vegetation change, would obligate CAM plants become “facultative” if biotic competition is too strong? If climate change creates more extreme seasonality, hence more environmental stress to facultative CAM plants, would they adapt successfully with their phenotypic flexibility, or be limited by the disadvantage in their photosynthetic pathway? Overall, a multifaceted network controls the CAM induction in plants and thus provides many directions for future studies on this subject.

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Please see online supplementary material for References.