# Specific leaf areas of the tank bromeliad *Guzmania monostachia* perform distinct functions in response to water shortage

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## ARTICLE INFO

## ABSTRACT

Leaves comprise most of the vegetative body of tank bromeliads and are usually subjected to strong longitudinal gradients. For instance, while the leaf base is in contact with the water accumulated in the tank, the more light-exposed middle and upper leaf sections have no direct access to this water reservoir. Therefore, the present study attempted to investigate whether different leaf portions of *Guzmania monostachia*, a tank-forming $C_3$-CAM bromeliad, play distinct physiological roles in response to water shortage, which is a major abiotic constraint in the epiphytic habitat. Internal and external morphological features, relative water content, pigment composition and the degree of CAM expression were evaluated in basal, middle and apical leaf portions in order to allow the establishment of correlations between the structure and the functional importance of each leaf region. Results indicated that besides marked structural differences, a high level of functional specialization is also present along the leaves of this bromeliad. When the tank water was depleted, the abundant hydrenchyma of basal leaf portions was the main reservoir for maintaining a stable water status in the photosynthetic tissues of the apical region. In contrast, the CAM pathway was intensified specifically in the upper leaf section, which is in agreement with the presence of features more suitable for the occurrence of photosynthesis at this portion. Gas exchange data indicated that internal recycling of respiratory $CO_2$ accounted for virtually all nighttime acid accumulation, characterizing a typical CAM-idling pathway in the drought-exposed plants. Altogether, these data reveal a remarkable physiological complexity along the leaves of *G. monostachia*, which might be a key adaptation to the intermittent water supply of the epiphytic niche.

## Introduction

The epiphytic habitat represents a highly dynamic environment, subject to temporal and spatial variations in irradiation, nutrient and water supply. Among these abiotic factors, sporadic or seasonal periods of water shortage are perhaps one of the most common challenges even for epiphytes occurring in humid tropics, which are characterized by a high annual rainfall (Zotz and Thomas, 1999). Some of these epiphytes, such as the bromeliads, lack an absorptive root system and completely depend on direct precipitation for their water supply (Benzing, 1990). Therefore, a suite of adaptations must be employed by these plants in order to cope with an intermittent water supply (Zotz and Hietz, 2001).

Approximately half of all bromeliads are epiphytic, and the success of this family in the epiphytic niche is frequently associated with the development of strategies to intercept, absorb and store rainwater more efficiently (Benzing, 2000). In the tank-forming bromeliads, for example, the rainwater accumulates in external tanks (phytotelma) formed by the overlapping of the leaf bases, allowing the plant to draw upon their water reservoir during periods of drought (Schmidt and Zotz, 2001). Moreover, the presence of epidermal trichomes on the leaf surfaces ensures an efficient way for water absorption in most of these epiphytic bromeliads (Benzing, 2000).

In addition to these morphological specializations, a large number of epiphytic bromeliads display Crassulacean Acid

Metabolism (CAM), which is a specialized photosynthetic pathway that minimizes the plant evaporative demand by opening its stomata mainly throughout the night when atmospheric vapor pressure deficits are lower (Lüttge, 2004). In fact, it is well-known that CAM bromeliads are especially abundant in epiphytic communities of dry forests (Griffiths and Smith, 1983), which reinforces the ecological importance of the CAM pathway for the survival of vascular epiphytes in water-limited environments.

Besides water economy, another key feature of the CAM is the remarkable plasticity provided by this mode of photosynthesis, especially among the $C_3$-CAM facultative species, which can perform either $C_3$ or CAM depending on the environmental conditions (Cushman, 2001). Among the bromeliads, the best recognized facultative CAM species is Guzmania monostachia (Medina et al., 1977; Griffiths and Smith, 1983; Maxwell et al., 1992). This epiphytic tank bromeliad is widespread throughout the middle to upper canopy in neotropical forests and shows a high capacity of acclimation in response to variations in the natural environment (Maxwell et al., 1994).

Water availability and light intensity seem to be the major environmental factors controlling the degree of CAM expression in G. monostachia (Medina et al., 1977; Maxwell et al., 1994). Medina et al. (1977) observed that this bromeliad exhibits atmospheric $CO_2$ uptake only during the day with a small day/night malate fluctuation when water is not limited. On the other hand, after approximately one week of water shortage, the $CO_2$ uptake occurred predominantly during nighttime, and larger acid fluctuations were observed. Moreover, re-watering drought-exposed G. monostachia plants resulted in a reversible shift from CAM to $C_3$ photosynthesis (Medina et al., 1977).

By analyzing the gas exchange of G. monostachia growing as an epiphyte, Lüttge et al. (1986) found that nocturnal recycling of respired $CO_2$ is particularly important under field conditions. Afterwards, studies carried out by Maxwell et al. (1994) also indicated that the nocturnal acid accumulation in drought-exposed G. monostachia resulted mainly from re-fixation of respiratory $CO_2$. This nocturnal regeneration of respiratory $CO_2$ can be considered an important CAM response to severe stress conditions since the removal of this internal $CO_2$ source significantly increased the susceptibility to photoinhibition as demonstrated for Pyrrosia piloselloides (Griffiths et al., 1989).

Typical for most bromeliads, G. monostachia exhibits long-lived leaves arranged in rosettes (Griffiths and Smith, 1983). This rosette-habit creates a longitudinal light gradient along the leaf as the top portion receives more light than the leaf bases during the entire life span (Popp et al., 2003). Moreover, in leaves of tank-forming bromeliads there is the establishment of additional gradients along the leaf blade. In these plants, only the leaf bases (also called leaf sheaths) are in direct contact with the water accumulated in the tank and have the ability to absorb this resource (Schmidt and Zotz, 2001). On the other hand, the presence of water in the tank also limits the capacity of the leaf bases to exchange gas with the atmosphere (Benzing, 2000).

Consequently, we should expect strong functional differences along the leaves of bromeliads, especially among the tank-forming species. However, only few studies have analyzed the existence of physiological gradients along the bromeliad leaves. In the CAM species Aechmea aquilega, for instance, the highest values of nocturnal acid accumulation ($\Delta H^+$) and $CO_2$ uptake were found at the distal third of the leaf (Lüttge et al., 1986). Also, significant differences in the content of nitrogen and lipids and in the $\delta^{13}C$ values were detected between the chlorophyll-containing tissues and the non-chlorophyllous leaf bases of pineapple leaves (Medina et al., 1994). Further, Popp et al. (2003) observed that the leaf bases of five CAM bromeliads possessed the lowest

levels of $\Delta H^+$ when compared to their middle and upper leaf portions.

Based on these studies, the present work was designed to evaluate whether different leaf portions of G. monostachia, a tank-forming $C_3$-CAM bromeliad, play distinct physiological roles during the modulation of the photosynthetic pathway in response to changes in water availability. The level of CAM was evaluated in basal, middle and apical leaf portions of this bromeliad by analyzing the changes in $\Delta H^+$ and in the activities of key enzymes of the CAM pathway. Additionally, internal and external morphological features, relative water content and chlorophylls and carotenoids levels were analyzed in these portions in order to allow the establishment of correlations between the structure and the functional importance of each leaf region. Finally, gas exchange analyses were performed in the leaf portions to obtain more information about possible differences in the photosynthetic capacity along the leaf blade.

## Material and methods

### Plant growth and treatments

Adult plants of Guzmania monostachia (L.) Rusby ex Mez var. monostachia (see Supplementary Table S1 for growth stage characterization) were taken from stocks maintained in a greenhouse of the Department of Botany at the University of São Paulo, São Paulo, Brazil, and transferred to controlled environment chambers.

Throughout the experiments, and for the preceding three weeks of acclimation, the plants were maintained in a growth chamber at a photosynthetic flux density (PFD) of about $250\,\mu mol\,m^{-2}\,s^{-1}$ supplied by fluorescent lamps (Sylvania, Germany), 12 h photoperiod, day/night air temperature of $25/20\,°C$, and day/night relative humidity of 60/70%. PFD inside the growth chamber was monitored with an LI-190 quantum sensor connected to an LI-250 A meter (LI-COR Instruments, USA). All plants were cultivated in pots containing vermiculite, with one plant per pot. They were watered with distilled water on a daily basis and received a 10% (v:v) dilution of Hoagland's solution once a week (Hoagland and Arnon, 1938).

After the acclimation period, plants were separated into three experimental groups, each one submitted to a different condition of watering: (a) control plants watered daily for a week, (b) water stress treatment, in which water was withheld for a week and (c) rewatered treatment, which consisted of plants subjected to drought for a week and, then, watered daily for another week.

### Leaf sampling

After each treatment, the 2nd to the 8th youngest fully developed leaves from five individual plants were collected 1 h after the onset of illumination and divided in three portions: (a) basal, corresponding to the part of the leaf that forms the tank; (b) middle, corresponding to the lower half of the green portion of the leaf blade; and (c) apical, corresponding to upper half of the green part of the leaf blade. All samples corresponding to each leaf portion were fragmented into small pieces of about 5 mm length, weighed, frozen in liquid nitrogen and stored at $-20\,°C$ until use in the biochemical analyses. Additional samples for titratable acidity determination were taken 1 h before the end of the light period, frozen in liquid nitrogen and also stored at $-20\,°C$ until use.