

ORIGINAL RESEARCH

Water exchange between the Chlorenchyma and the Hydrenchyma and its physiological role in leaves with Crassulacean acid metabolism

Paulo José Vieira Cabrita 

IAPN – Institute of Applied Plant Nutrition,
Georg-August-University Göttingen,
Göttingen, Germany

Correspondence

Paulo José Vieira Cabrita,
Email: paulo.cabrita@agr.uni-goettingen.de

Edited by J. Flexas

Abstract

Direct and non-destructive measurements of plant-water relations of plants exhibiting the Crassulacean acid metabolism (CAM) photosynthetic pathway are seldom addressed, with most findings inferred from gas exchange measurements. The main focus of this paper was to study how the water exchange between the chlorenchyma and the hydrenchyma depends on and follows the CAM photosynthetic diel pattern using non-invasive and continuous methods. Gas exchange and leaf patch clamp pressure probe (LPCP) measurements were performed on *Aloe vera* (L.) Burm f., a CAM species, and compared to measurements on banana (*Musa acuminata* Colla), a C₃ species. The LPCP output pressure, P_p , of *Aloe vera* plants follows its diel CAM photosynthetic cycle, reversed to that observed in banana and other C₃ species. The four phases of CAM photosynthesis can also be identified in the diel LPCP output pressure, P_p , cycle. The P_p values in *Aloe vera* are determined by the hydrenchyma turgor pressure, with both parameters being reversely related. A non-invasive and continuous assessment of the water exchange between the chlorenchyma and the hydrenchyma in CAM plants, namely, by following the changes in the hydrenchyma turgor pressure, is presented. However, showing once more how the LPCP output pressure, P_p , depends on the leaf structure, such an approach can be used to study plant-water relations in other CAM species with a leaf structure similar to *Aloe vera*, with the hydrenchyma composing most of the leaf volume.

1 | INTRODUCTION

The Crassulacean acid metabolism (CAM) photosynthetic pathway has been observed on succulent plants of semiarid terrestrial and epiphytic habitats, subject to limiting water availability (Zotz and Hietz, 2001), as well as some aquatic plants living under limiting carbon dioxide conditions (Herrera, 2009; Lüttge, 2004). So far, they have been identified in 37 plant families (Winter et al., 2021), but this is likely to increase as more species between obligate and facultative CAM modes are found. Some CAM species are of great economic

importance, with the most valuable belonging to the genera *Agave*, *Aloe*, *Ananas*, *Opuntia*, and *Vanilla* (Davis et al., 2019). Common to all species on which it has been observed, the CAM photosynthetic pathway is mostly distinguished by the nocturnal fixation of CO₂ via cytoplasmic phosphoenolpyruvate carboxylase (PEPC) and the subsequent accumulation of organic acids in the vacuole, mostly malic acid, but also citric acid in some cases (Lüttge, 1988, 2006). The stored organic acids are diurnally decarboxylated, resulting in the release of CO₂ and its fixation by rubisco in the Calvin cycle, similar to C₃ plants, during the day. The diel cycle of the CAM photosynthetic pathway can be

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Physiologia Plantarum* published by John Wiley & Sons Ltd on behalf of Scandinavian Plant Physiology Society.

divided into four phases (Osmond, 1978). During the night, CAM plants transpire and take up CO_2 fixed via PEPC, in what is called phase I (the carboxylation phase). In this phase, the water vapour pressure deficit (VPD) between the plant transpiring surfaces and the surrounding air reaches its lowest value, thus reducing water loss by transpiration. The fixed carbon is used to synthesise organic acids in the cytosol, subsequently stored in the vacuole, leading to the acidification of the chlorenchyma. Therefore, as the night advances, the concentration of stored organic acids increases, reaching its maximum while the rate of CO_2 -uptake and carboxylation decreases.

As the photoperiod begins, with the stomata still open and the organic acids accumulating in the vacuole, CO_2 -fixation by PEPC is progressively reduced until it is taken over by the fixation by rubisco. This change characterizes the transient phase II, during which higher rates of CO_2 -uptake can be observed, concomitant with an increase in total water vapour conductance. However, these sudden changes are mostly observed following abrupt changes in light under laboratory conditions (Lambers et al., 2008). Under field conditions, the duration of phase II can be longer (Ball et al., 1991; Lee et al., 1989). These transient peaks in CO_2 -uptake and total water vapour conductance are brought up by the combination of the increase in temperature and VPD that happen concomitantly with a sudden increase in light intensity (e.g. by turning on the lights in the greenhouse), leading to a sudden increase in rubisco activity and transpiration. During the photoperiod with stomata closed, triggered by the very high internal concentration of CO_2 resulting from the decarboxylation of the organic acids accumulated in the vacuole overnight, CAM plants perform the Calvin cycle and accumulate carbohydrates in phase III. During this diurnal phase of CAM photosynthesis, as the intercellular CO_2 concentration is increased considerably, suppressing photorespiration, the amount of organic acids stored in the vacuole is reduced progressively until it is eventually completely depleted. This leads to the intercellular CO_2 concentration dropping considerably to low levels, triggering stomata to open and take up CO_2 as the end of the photoperiod approaches, in what defines the beginning of phase IV. Consequently, in this phase, there is a gradual increase in the CO_2 -uptake reflecting the concomitant fixation of exogenous CO_2 by rubisco. Afterwards, as the night starts, so does phase I and the fixation of CO_2 by PEPC, which may have started already during late phase IV, when the rubisco activity decreases with light as the photoperiod ends. Essentially, in the same way as the C_4 photosynthetic pathway, CAM is a strategy that evolved in some species to concentrate CO_2 around rubisco, thereby increasing their photosynthetic performance and water-use efficiency under adverse environmental conditions (Edwards and Ogburn, 2012).

To optimize photosynthesis and live under such unfavourable ambient conditions, terrestrial CAM plants developed specific morphological and anatomical features, including low surface-area-to-volume ratio (SA:V); thick cuticle; low density of small stomata; large vacuoles occupying most of the cell volume; mesophyll cells tightly packed; and specialized achlorophyllous water-storage parenchyma (also called hydrenchyma), most noticeable in many succulent plants (Borland et al., 2018; Nelson et al., 2005; Ogburn and Edwards, 2010).

These features minimise water and CO_2 losses during the day, resulting in low transpiration rates and total water vapour conductance (Nelson et al., 2005; Silva et al., 2014). A permanent high plant water status in terrestrial CAM plants is required for the nocturnal accumulation of organic acids in the chlorenchyma, decreasing the cell osmotic potential and maintaining high water potential in their tissues during the photoperiod. This is possible due to their succulence, i.e., the capacity to store large amounts of withdrawable water in their bodies. In terrestrial CAM plants, water is stored either in expanded chlorenchyma cells, with very large vacuoles, in what is called all-cell succulence (e.g., *Kalanchoe*), or in the hydrenchyma, known as storage succulence (e.g., *Aloe*, *Agave*, *Lithops*) (Borland et al., 2018; Ogburn and Edwards, 2010; Smith and Lüttge, 1985; Steudle et al., 1975).

Like in all plants, water movement and distribution in CAM species are driven by water potential differences between different regions of the plant body. Water moves from the hydrenchyma to the chlorenchyma driven by the nocturnal decrease in the chlorenchyma water potential caused by the decrease of its osmotic potential due to the accumulation of organic acids in the vacuoles in phase I. Therefore, the chlorenchyma turgor pressure is increased despite the concomitant decrease in its water potential (Lüttge and Nobel, 1984; Rygol et al., 1986; Smith et al., 1987). As the organic acids accumulated in the chlorenchyma are decarboxylated and eventually depleted during the photoperiod, thereby increasing its water potential, at some point during phase III, the water potential difference between the chlorenchyma and the hydrenchyma is reversed, driving water back to the hydrenchyma, increasing its turgor pressure. Therefore, coupled with the diel cycle of the CAM photosynthetic pathway, there is also a diel cycle of water movement and distribution between the chlorenchyma and the hydrenchyma in succulent CAM plants, buffering the water status of the former. To this refilling of the hydrenchyma, water coming from the soil is also added (Goldstein et al., 1991; Schulte and Nobel, 1989; Schulte et al., 1987; Smith et al., 1989; Tissue et al., 1991). The water exchange between the chlorenchyma and the hydrenchyma is an entirely passive process with no associated energy costs. Up to 50% of the nocturnal transpiration in terrestrial CAM plants can be supplied only by the hydrenchyma water reserves. Under extreme drought, as observed in deserts, with soil water uptake being ceased completely, water released from the hydrenchyma can support the transpiration for many days (Nobel, 1976, 1977).

The physiological mechanism by which succulent plants store water in specialized cells is determined by the cell hydraulic capacitance, C ($\text{m}^3 \cdot \text{Pa}^{-1}$), that can be defined by the change in cell volume per unit of change of water potential (Nobel, 1983) or turgor pressure (Smith et al., 1987). At the whole-plant level, the hydraulic capacitance determines the buffering of water flow through the plant body by the water storage tissues (Leverett et al., 2023; Ogburn and Edwards, 2010). The hydraulic capacitance is thus dependent on the elastic properties of cell walls in accommodating such changes in cell volume by changing turgor pressure. Ultimately, it is the cell volumetric elastic modulus ϵ (Broyer, 1952; Philip, 1958) that determines the cell hydraulic capacitance, as it characterizes the expandability of a plant cell, i.e., how easily the plant cell volume changes by the build-up of

pressure in it. The hydraulic capacitance of succulent plant cells or tissues, such as the hydrenchyma, tends to be higher than that of non-succulent tissues, which explains the ability of these plants to maintain turgor, relative water content, and relatively high values of water potential during desiccation (Ogburn and Edwards, 2010). The volume of these cells will vary greatly, depending on the amount of water taken up or lost for a given change in water potential compared to cells with smaller hydraulic capacitance. Therefore, hydrenchyma cells have smaller values of the cell volumetric elastic modulus ϵ , being less rigid than their adjacent chlorenchyma cells (Goldstein et al., 1991; Ogburn and Edwards, 2010). Under extreme drought situations, chlorenchyma cells stay hydrated and functional at the expense of hydrenchyma cells, losing up to 85% of their water content. This is achieved in part by removing solutes from the hydrenchyma, thus adjusting its osmotic potential and increasing its water potential relative to the chlorenchyma, consequently favouring water movement into it (Barcikowski and Nobel, 1984; Schmidt and Kaiser, 1987).

The main focus of this paper was to study the influence of the CAM photosynthesis rhythm of the chlorenchyma on the plant-water relations of terrestrial CAM plants non-invasively and continuously. Namely, how the water exchange between the chlorenchyma and the hydrenchyma depends on and follows the CAM photosynthetic diel pattern of the former. Considering the presence of a voluminous hydrenchyma on most terrestrial CAM plant leaves and the principle behind the leaf patch clamp pressure probe (LPCP) (Cabrita, 2022; Zimmermann et al., 2008, 2013), it was hypothesized that the changes in its output pressure, P_p , reflected changes in the hydrenchyma turgor pressure, P_c . Although confirming previous works that showed the reverse relationship between the two parameters on different plant species (e.g., Ehrenberger et al., 2012; Martínez-Gimeno et al., 2017; Riboldi et al., 2016; Rüger et al., 2010a, b; Zimmerman et al., 2010, 2013), the novelty brought by the work here presented was that it allowed to assess changes in the hydrenchyma turgor pressure specifically and relate its diel pattern with CAM photosynthesis. Confirming the findings of Cabrita (2022), this is one more example of how the interpretation of the LPCP output pressure, P_p , depends on the leaf structure on which the probes are applied. Gas exchange and LPCP measurements were done on *Aloe vera* (L) Burm f. plants, a typical CAM species, and compared against those made on banana (*Musa acuminata* Colla), a C_3 plant. Although direct measurements of the turgor pressure on individual hydrenchyma cells have been reported before (Rygor et al., 1986; Steudle et al., 1975), a non-invasive and continuous approach to measure and assess changes in the hydrenchyma turgor pressure as well as identifying and monitoring CAM photosynthesis through plant-water relations is thus presented.

2 | MATERIALS AND METHODS

2.1 | Plants

Measurements were performed on *Aloe vera* (L.) Burm f. and banana (*Musa acuminata* Colla (AAA group) 'Dwarf Cavendish') plants growing

in the greenhouse at IAPN – Institute of Applied Plant Nutrition, in Göttingen, Germany. Plants were grown in 6 L pots using the soil mix Fruhstorfer Erde Typ T Struktur 1b (HAWITA GRUPPE GmbH), designed for ornamental plants, watered, and fertilized regularly with a N:P:K ratio of 2.5:1:2 (Universal-Flüssigdünger, Schmees GmbH). Temperature, relative humidity, and air pressure were continuously recorded (Web-Thermo-Hygrobarograph, Wiesemann & Theis GmbH), under a 16/8 h light/dark regime supplied by high-pressure sodium-vapor lamps (Philips MASTER AGRO 400 W). At 1 m above ground during the light period, the photosynthetic photon flux density (PPFD) varied between approximately 150 and 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Withholding watering was used to impose water deficit, while the effects of transient changes in the ambient parameters were studied on plants growing in a 52.8 m³ plant growth chamber (Weiss Technik UK Ltd.) under 25/18°C temperature and 60/70% relative humidity in a 16/8 h light/dark regime supplied by LED illumination (Valoya LED Grow Lights B200 NS1 + FR, Valoya Oy.) providing a photosynthetic photon flux density (PPFD) of 315 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy height approximately.

2.2 | Leaf patch clamp pressure probe measurements

Plant-water relations were studied by applying leaf patch clamp pressure probes (LPCP) on the leaves. The resulting output pressure, P_p , was measured using a set of sensors and associated components for telemetric and mobile network-based data transfer to the internet (YARA ZIM Plant Technology GmbH, Hennigsdorf, Germany). Unless otherwise specified, the magnetic pad containing the pressure sensor was applied on the abaxial leaf surface, and data was recorded with a 1-minute sampling interval. The relaxation time constants of the changes in the output pressure, P_p , values during the transition between the light periods, τ_{on} (when lights were turned on) and τ_{off} (when lights were turned off), were determined following Cabrita (2022) taking into consideration the first 60 min after lights were turned on and off, respectively.

2.3 | Gas exchange measurements

The photosynthetic parameters CO_2 net assimilation rate (A), total water vapour conductance ($g_{\text{H}_2\text{O}}$), leaf-to-air vapour pressure deficit (VPD), and transpiration (E) were measured with a portable open gas-exchange fluorescence system GFS-3000-C, equipped with a high-precision $\text{CO}_2/\text{H}_2\text{O}$ infrared gas analyser and a Standard Measuring Head 3010-S (Heinz Walz GmbH). Plant leaves were enclosed in an 8 cm² leaf chamber (cuvette), in which the environmental parameters were set to follow the surrounding ambient conditions (temperature, relative humidity, and light). The flow rate was set to 750 $\mu\text{mol s}^{-1}$, and measurements commenced with a 1-minute sampling interval, following stability in the cuvette conditions, which were normally achieved after 2 to 3 min of enclosing the leaf in the cuvette approximately.

2.4 | Statistical Analysis

Statistical analyses were conducted using Microsoft Excel (2019), in which one-way analysis of variance (ANOVA) and the Duncan test ($p > 0.05$) were used to determine the differences between means. Parameter differences, as suggested by ANOVA results, were thus appropriately referenced in the text.

3 | RESULTS

3.1 | The diel pattern of the LPCP output pressure P_p in *Aloe vera* leaves is opposite to that of banana

As Figure 1 shows, the typical diel changes of the LPCP output pressure, P_p , of *Aloe vera* leaves (Figure 1A) were opposite to those observed on banana leaves (Figure 1B), including the plants' responses to changing the lighting in the greenhouse. That is, the highest values of the output pressure P_p in *Aloe vera* leaves were observed during the dark period and its lowest values around halfway through the light period, when the temperature was highest, and the relative humidity was lowest (Figure 1C). In banana, following its photosynthetic diel cycle, P_p values were highest during the light period, when transpiration was highest due to higher leaf-to-air vapour pressure deficit (VPD), and decreased afterwards throughout the day, reaching its lowest when the stomata were mostly closed during the dark period (Figure 1B) and the relative humidity was highest (Figure 1C). The output pressure P_p in *Aloe vera* plants decreased after lights in the greenhouse were turned on and increased after they were turned off in the evening (Figure 1A), contrary to that observed on banana leaves (Figure 1B). Similar results were always observed on all plants of both species studied and no difference was made between applying the pad with the sensor chip on the leaf abaxial or the adaxial side (results not shown). The changes in the output pressure P_p as plants responded to changes in the greenhouse illumination can be well described by exponential functions (see Supplemental Material) characterized by corresponding relaxation time constants τ_{on} and τ_{off} , defined as:

$$\tau_i = \frac{1}{P_p} \left(\frac{\partial P_p}{\partial t} \right)_i, i = \text{on, off} \quad (1)$$

Where subscripts on and off refer to the time interval when lights in the greenhouse were turned on and off, respectively. The abrupt changes in the greenhouse illumination caused transient changes in ambient parameters, namely, temperature and relative humidity, to which plants had to adapt (Figure 1C). Upon turning on and off the lights, there was an exponential change in the P_p values of both species that could be still observed during the immediate 60 min afterwards (see Supplemental Material). For the 5 days considered in Figure 1, during which plants were kept under normal water supply, the relaxation time constants observed on banana were $\tau_{on} = 0.8 \pm 0.1 \times 10^{-3} \text{ min}^{-1}$ ($R^2 > 0.94 \pm 0.03$, $n = 15$) and $\tau_{off} = -0.7$

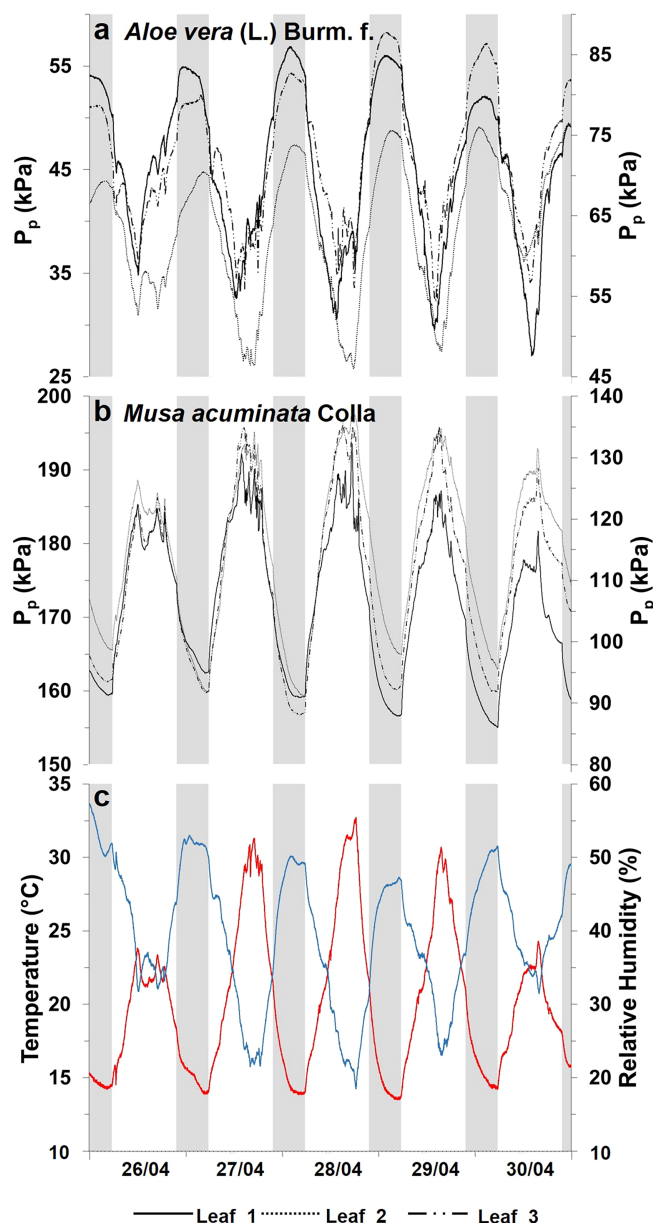


FIGURE 1 Diel pattern of the leaf patch clamp pressure probe (LPCP) output pressure, P_p . Measurements were made on (A) three leaves of a *Aloe vera* plant, of which the P_p values of Leaf 3 are referenced to right vertical axis, and on (B) three leaves of a banana plant, with the P_p values of Leaf 2 referenced to right vertical axis, under (C) the corresponding diel changes of temperature (red line) and relative humidity (blue line). Dark period (8 h) indicated by the grey shaded areas. For the sake of clarity and better understanding of the similar patterns of the diel changes in P_p observed, referencing to the right vertical axis was thus decided.

$\pm 0.1 \times 10^{-3} \text{ min}^{-1}$ ($R^2 > 0.98 \pm 0.01$, $n = 15$). In *Aloe vera* one obtained $\tau_{on} = -1.5 \pm 0.1 \times 10^{-3} \text{ min}^{-1}$ ($R^2 > 0.87 \pm 0.04$, $n = 15$) and $\tau_{off} = 1.1 \pm 0.1 \times 10^{-3} \text{ min}^{-1}$ ($R^2 > 0.95 \pm 0.01$, $n = 15$). While for each species, the relative changes in the output pressure P_p with time, Equation (1), during the transition between light periods with its consequent changing of the surrounding ambient conditions

(Figure 1C), were similar in magnitude, the relative changes were higher in *Aloe vera* than in banana. Such differences in the relaxation time constants between both species were always observed on all plants studied under normal irrigation.

However, under drought stress conditions, the responses of both species to irrigation regarding the changes in the LPCP output pressure P_p with time during the transition between the light and dark periods, described by relaxation time constants (Equation (1)), were different (Figures 2, 3). As Figure 2 shows, the relaxation time constants τ_{on} and τ_{off} of *Aloe vera* remained basically stable and did not seem to change due to irrigation, even after longer periods between irrigations. That is, the relative change in the output pressure P_p with time during the transition between the light and the dark periods, Equation (1), remained basically the same, independently of the balance that plants had to keep between the availability of water in the

soil and the water demand to sustain physiology and transpiration. Such pattern was not observed on banana, as Figure 3 clearly illustrates. As the soil water availability decreased with time, so did the magnitude of the relaxation time constants τ_{on} and τ_{off} in banana. That is, the relative changes in output pressure P_p with time, Equation (1), became smaller with decreasing soil water availability. Once plants were irrigated, the values of τ_{on} and τ_{off} increased in magnitude, reflecting a higher relative change of the output pressure P_p with time after changes in the greenhouse illumination (Figure 3). Considering the well-known relationship between the LPCP output pressure, P_p , and the leaf patch turgor pressure, P_c , that is also observed on banana, these results suggest that an improvement on the overall plant water status, resulting from irrigation, induces faster responses from the plants to the changing ambient conditions whenever lights in the greenhouse are turned on and off. That is, from the relationship between the LPCP output pressure, P_p , and the leaf patch turgor pressure, P_c , observed in previous works:

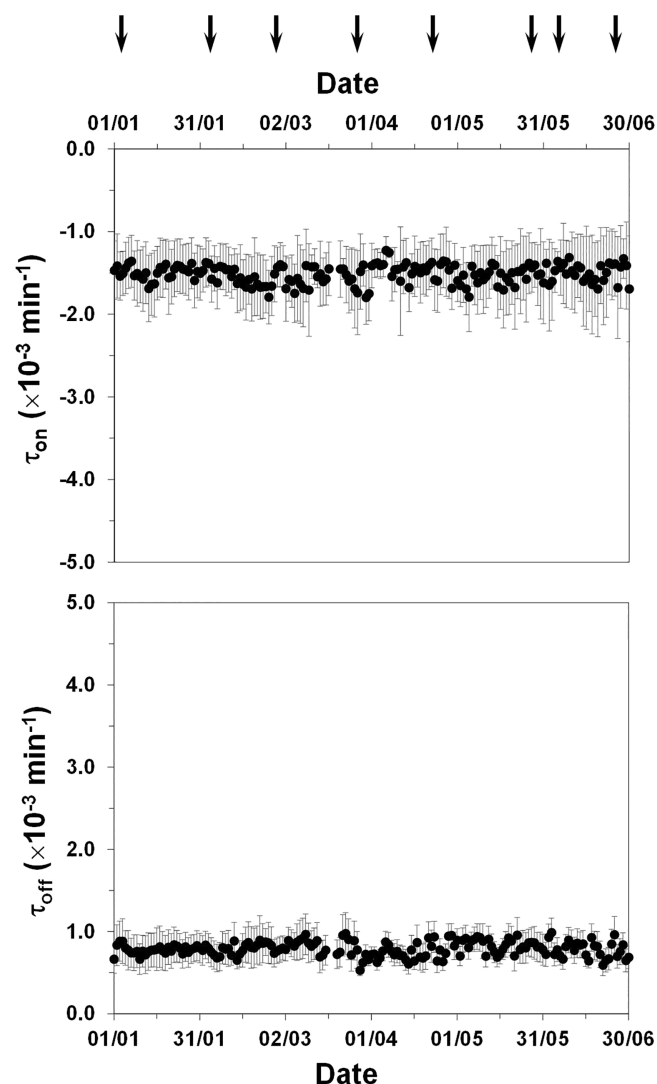


FIGURE 2 Relaxation time constants τ_{on} and τ_{off} of *Aloe vera*. Measurements were done during the first 60 min after turning on and off the greenhouse lights, as indicated by the respective subscripts. Irrigations are indicated by arrows. Mean values $\pm$ SE ($n = 9$, $p \leq 0.05$).

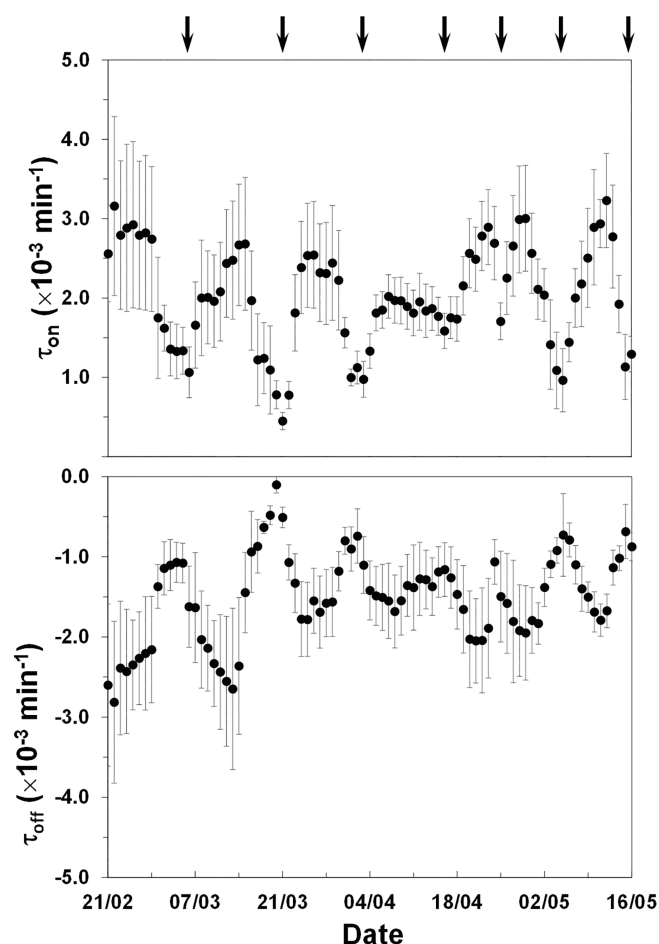


FIGURE 3 Relaxation time constants τ_{on} and τ_{off} of banana. Measurements were done as indicated in Figure 2. Arrows indicating irrigations. Mean values $\pm$ SE, for the sake of clarity and graphic presentation, significant differences between values were omitted ($n = 9$, $p \leq 0.05$).

$$P_p = \frac{K}{F(P_c)} P_{clamp} \quad (2)$$

where P_{clamp} , the pressure applied by the two magnets clamped to a leaf, constant K , and F , as an increasing function of the leaf patch turgor pressure, P_c , are known, Equation (1) can be written as:

$$\tau_i = -\frac{1}{F(P_c)} \frac{\partial F}{\partial P_p} \left(\frac{\partial P_c}{\partial t} \right)_i, i = \text{on, off} \quad (3)$$

The LPCP output pressure, P_p , varies opposite to the leaf patch turgor pressure, P_c , which, in banana, is lowest under higher VPD values and highest in the dark period under lowest VPD conditions. The form and dependence of function F on P_c does not change in irrigated plants grown under normal ambient conditions, as shown in previous works. This suggests that higher values of $\frac{dP_p}{dt}$ also reflect bigger changes in the leaf turgor pressure, P_c , with time. In other words, after irrigation and with a replenished water status, higher values of the relaxation time constants τ_{on} and τ_{off} reflect bigger changes in the leaf turgor pressure, P_c , as plants adapt to changes in transpiration caused by changes in the ambient parameters during the fast transition between the light and dark periods in the greenhouse (Equation (3)). Ultimately, the relaxation constants τ_{on} and τ_{off} describe how fast plants distribute water throughout their tissues in order to respond to changes in the environment surrounding them (Figure 1C). Such distribution of water between different plant tissues not only depends on the balance between water availability in the soil and transpiration but also on the hydraulic conductance between those different tissues. In the case presented here, τ_{on} and τ_{off} reflect the hydraulic conductance within leaf tissues. As time passes and the water availability in the soil decreases between irrigations, the plants' fitness in responding to such diel changes in ambient parameters decreases as reflected in the decrease in the magnitudes of τ_{on} and τ_{off} (Figure 3), i.e., in the magnitude of $\frac{dP_c}{dt}$ (Equation (3)). Nevertheless, if Equation (2) is also expected to be observed on *Aloe vera* in the same way as on banana, the results of Figure 2 suggest that the distribution of water within *Aloe vera* leaves is hardly unaffected by changes in water availability in the soil under conditions that otherwise greatly affect species like banana. Such differences in the water distribution within leaf tissues and, consequently, in their hydraulic conductance between both species, as suggested by these results, seem highly plausible considering their different leaf structure; particularly the presence of a specialized water storage parenchyma composing a big fraction of the volume in *Aloe vera* leaves.

The effects of the changes in the relaxation time constants τ_{on} and τ_{off} in banana under drought stress (Figure 3) on the values of LPCP output pressure, P_p , with time are shown in Figure 4. With the prolonging decrease of the soil water availability, the values of the output pressure P_p (Figure 4A) as well as the difference between its diel maximum and the minimum values, ΔP_p (Figure 4B), reached during the light and the dark period respectively, increased with time between irrigations initially. Considering Equation (2), these results indicate that under drought stress, there is a decrease in leaf turgor

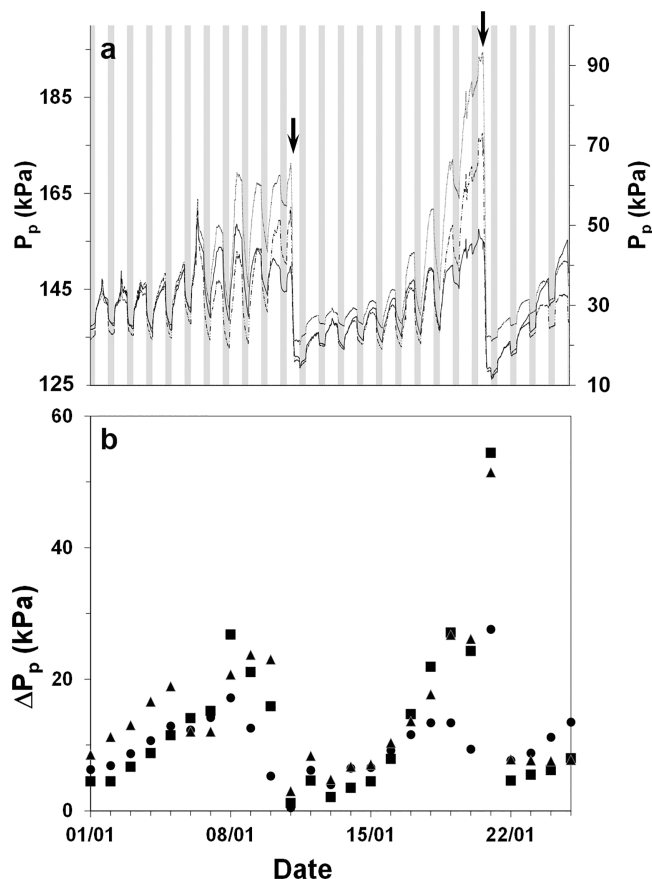


FIGURE 4 Leaf patch clamp pressure probe (LPCP) output pressure, P_p , in banana under drought stress, (A) diel patterns of three leaves, with P_p values of Leaf 3 referenced to the right vertical axis and (B) corresponding daily difference between maximum and minimum P_p values, with the different symbols representing the different leaves, i.e., circle, square, and triangle as leaves 1, 2, and 3, respectively. Leaves in (A) are distinguished as in Figure 1. Arrows indicating irrigations. Dark period, as indicated in Figure 1.

pressure, P_c , with an increase in the difference between its diel maximum and minimum values, ΔP_c , in banana, as the plant tries to keep up the balance between decreasing soil water availability, transpiration, and physiological water demand. However, the fact that such an increasing trend stops at some point during drought stress and ΔP_p starts to decrease with time, albeit differently between leaves (Figure 4B), suggests that the plant's fitness in keeping such balance may be approaching its limits. That is, with less water being available with time, the plant's ability to redistribute it efficiently at the end of each diel photosynthetic cycle between different tissues is compromised, resulting in approaching levels of turgor pressure between day and night extremes. That is also suggested by the decreased magnitude of the relaxation time constants just before irrigations (Figure 3), suggesting difficulties in redistributing water efficiently throughout its leaves and controlling turgor pressure efficiently. Upon irrigation, both P_p and ΔP_p decrease sharply (Figures 4A, B), suggesting a concomitant increase in leaf turgor pressure, P_c , as one expects after the reestablishment of a normal plant water status. The differences between the

leaves shown in Figure 4A, B might be related not only to their age and physiological state but also their position in the plant body while the plant tries to adapt to decreasing water availability in the soil, transpiration, and the physiological demand on sustaining growth and development under such challenging conditions. It suggests that regarding redistribution of water and maintenance of water status, some priorities between leaves may be at play, e.g., younger vs older tissues. All combined, these factors determine the distribution of water between different regions of the plant body and, in this case, its leaves. In addition, as P_p and ΔP_p increase with time between irrigations (Figures 4A, B), this means that the relative change in P_p values with time during the transitions between light periods also decreases in magnitude, i. e., the relaxation time constants, Equation (1). Respectively, the output pressure, P_p , diel patterns shown in Figure 4A suggest a decrease in magnitude of the banana relaxation time constants τ_{on} and τ_{off} between irrigations. As time passes between irrigation, the smaller difference between the highest and lowest P_p values at each diel cycle indicates that the changes in P_p values with time also become smaller as they occur between increasing values that approach each other. This means that there is a pronounced decrease in $\frac{1}{P_p} \frac{dP_p}{dt}$, i.e., in the relaxation time constants as results in Figure 3 clearly illustrate. In stark contrast, although there are changes in P_p as well as ΔP_p values between different days, the patterns shown by banana (Figures 4A, B) were never observed on *Aloe vera*. In fact, no clear pattern of changes in P_p and ΔP_p was observed on *Aloe vera* plants between irrigations that could be related to changes in the soil water availability, VPD or temperature (results not shown).

3.2 | The CAM photosynthesis of *Aloe vera* leaves determines the changes in the LPCP output pressure P_p

As it is well-known, *Aloe vera* is a typical example of a plant showing CAM photosynthesis. Some physiological aspects associated with the diel changes in the assimilation rate, A , total water vapour conductance, g_{H_2O} (Figure 5A), and transpiration, E (Figure 5C), typical of the four phases of CAM photosynthesis identified in Figure 5A, seemed to be reflected in the LPCP output pressure, P_p , diel pattern of an irrigated *Aloe vera* plant (Figure 5B). Most obvious is the simultaneous nocturnal increase up to their respective maxima of the output pressure P_p (Figure 5B) together with the assimilation rate, A , total water vapour conductance, g_{H_2O} , and transpiration, E , (Figures 5A, C). Considering *Aloe vera* leaf structure, namely, the presence of a specialized water storage parenchyma (called hydrenchyma) occupying the vast majority of the leaf volume and its role in CAM photosynthesis, it is therefore reasonable to expect that an increase in the C_2O -uptake, A , (Figure 5A) leading to a decrease in the chlorenchyma osmotic potential in phase I would be reflected in an increase in P_p values (Figure 5B). As the chlorenchyma osmotic potential decreases due to the nocturnal accumulation of organic acids, water is drawn from the hydrenchyma causing its turgor pressure to decrease.

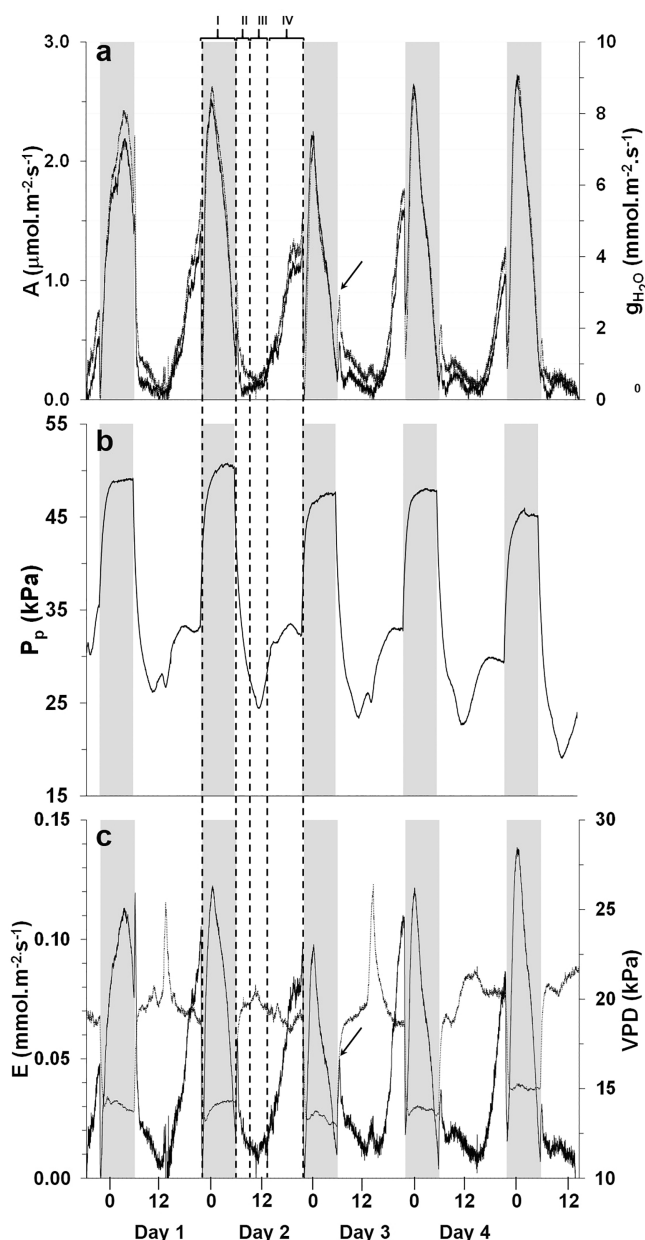


FIGURE 5 Photosynthetic activity and LPCP output pressure, P_p , diel pattern in *Aloe vera*: (A) assimilation rate, A , (black line) and total water vapour conductance, g_{H_2O} , (black dotted line); (B) LPCP output pressure, P_p ; (C) transpiration rate, E , (black line) and leaf-to-air vapour pressure difference, VPD, (black dotted line); all measured simultaneously. CAM photosynthesis phases are highlighted by their corresponding Roman numerals, respectively, and the peaks in the assimilation rate and total water vapour conductance typical of phase II are indicated by arrows. Dark period, as indicated in Figure 1.

Such a result would be further enhanced by the nocturnal transpiration under lower VPD (Figure 5C), as it is mostly sustained by the leaf hydrenchyma water reserves. These results are predicted by Equation (2) considering that the *Aloe vera* LPCP output pressure, P_p , is essentially determined by the changes in the hydrenchyma turgor pressure, which comprises most of the leaf volume,

i.e., the sensed leaf patch. Thus, this interpretation explains the nocturnal increase of the *Aloe vera* LPCP output pressure, P_p , observed experimentally (Figures 1A, 5B).

Such interpretation of the *Aloe vera* output pressure, P_p , diel pattern also explains the decreasing of the P_p values during the light period towards its minimum (Figures 5B) when the VPD is highest (Figure 5C) basically no transpiration is observed (Figures 5A, C). As explained above, during phase III, while performing the Calvin cycle, the refilling of the hydrenchyma is sustained by root water uptake as well as water coming from the chlorenchyma leading to an increase in its turgor pressure, i.e., a decrease in P_p values (Equation (2), Figures 1A, 5B). The very brief peaks in the photosynthetic parameters (A , E , g_{H_2O}) after lights are turned on are typical of phase II, as highlighted in Figures 5A and C, and are not reflected in the *Aloe vera* output pressure, P_p , diel pattern (Figure 5B). As mentioned above, the peaks in the assimilation rate (Figure 5A) occur due to the overtake of rubisco activity in taking up CO_2 compared to PEPC activity at the beginning of the light period while stomata are still open. Simultaneous with the surge in CO_2 -uptake of phase II (Figure 5A), promoted by a rapid increase in rubisco activity, there is also a surge in transpiration and total water vapour conductance (Figures 5A, C) due to the sudden increase in VPD (Figure 5C) caused by the change in the ambient parameters after lights are turned on. Nevertheless, such transient changes in the ambient parameters combined with the concomitant physiological effects seemed to be insufficient to disturb the water exchange between the chlorenchyma and the hydrenchyma in *Aloe vera* in terms of affecting the turgor pressure of the latter meaningfully, as results shown in Figure 5B suggest. By then, the chlorenchyma approaches its maximum storage capacity of organic acids, which will be used in the subsequent Calvin cycle that has just been initiated. Consequently, the water potential difference between the chlorenchyma and hydrenchyma reduces progressively towards its minimum, thus, decreasing the water flow from the hydrenchyma. As the chlorenchyma osmotic potential starts to increase, upon initiation of phase III, the water uptake from the hydrenchyma decreases until it is eventually reversed. The hydrenchyma starts its refill, thus increasing its turgor pressure and, consequently, decreasing the output pressure, P_p , Equation (2). Such result is well illustrated by the pronounced decrease in P_p values after lights are turned on (Figure 5B), which happens simultaneously with the transient phase II that marks the transition from the carboxylation phase I to the Calvin cycle (phase III) in CAM plants (Figures 5A, C). However, as explained above, it should be emphasized that this fast transition (phase II) is a consequence of the special context in which plants are kept.

Another aspect that favours the interpretation of the *Aloe vera* LPCP output pressure, P_p , diel pattern, as following the changes in the hydrenchyma turgor pressure reversely, is the increase in P_p values observed after its minimum is reached halfway through the light period. Once the reserves of organic acids accumulated in the chlorenchyma in phase I are depleted, stomata, triggered by the lowest levels of CO_2 in the chlorenchyma, are once again opened to supply rubisco with CO_2 to maintain phase III (Figures 5A, C). During this

second part of the light period and under relatively high VPD (Figure 5C), the subsequent transpiration is thus maintained by the hydrenchyma water reserves, which were refilled up to that time. Hence, under such conditions and after refilling, the hydrenchyma turgor pressure will start to decrease in the afternoon as the light period progresses, i.e., the output pressure P_p will thus increase simultaneously (Equation (2), Figure 5B). Due to the fast transition to the dark period, as lights are turned off, the rubisco activity stops, as temperature and VPD decrease (Figure 5C). Simultaneously, all these effects combined result in a sharp decrease in the assimilation rate, total water vapor conductance (Figure 5A), and transpiration (Figure 5C). Accordingly, such effects combined seemed to result in a slight decrease or stabilization of the LPCP output pressure, P_p , in *Aloe vera* (Figure 5B), reflecting the likely adaptation of the hydrenchyma in supplying transpiration under such conditions in the evening as the dark period begins. When that happens, PEPC activity resumes in taking up CO_2 (Figure 5A) under lower VPD and temperature, while more stomata open to supply its activity, thus increasing transpiration (Figure 5C). The synthesis and accumulation of organic acids in the chlorenchyma is thus revived, driving again water from the hydrenchyma. Consequently, there is an increase in the assimilation rate, total water vapour conductance, and transpiration after lights are turned off (Figures 5A, C). The hydrenchyma turgor pressure decreases, thus, increasing the output pressure P_p as shown in Figure 5B and predicted by Equation (2). As in phase II, the abrupt changes in the physiological parameters here described at the end of phase IV and beginning of phase I are again a direct consequence of the specific conditions under which plants are kept.

The different response of the LPCP output pressure, P_p , in both species to transient changes in ambient parameters during the light period (Figures 6, 7) not only derives from their different photosynthetic pathways but also further supports the interpretation that the diel pattern of *Aloe vera* output pressure, P_p , reflects the changes in its hydrenchyma turgor pressure reversely. The response of the output pressure, P_p , in banana to a transient drop in temperature and a simultaneous increase in relative humidity (Figure 6C) in the light period originates from their consequent effects on photosynthesis. Decreasing ambient temperature increases ambient relative humidity, i.e., decreases VPD, thus, consequently reducing transpiration. Simultaneously, the photosynthetic rate will also be reduced due to a decrease in rubisco activity as the temperature is lowered. Therefore, the water demand to keep such decreasing transpiration and build-up of photosynthates will be less. Additionally, lowering the temperature also reduces the mesophyll osmotic potential, decreasing its effect on the leaf water potential. All these effects combined lead to an increase in the mesophyll turgor pressure in banana. Hence, as predicted by Equation (2) and experimentally observed (Figure 6B), the LPCP output pressure P_p in banana decreases as temperature decreases until a new physiological state needed to sustain photosynthesis under cooler and more humid ambient conditions is reached. That can be easily seen by the maintenance of a lower output pressure P_p under a lower temperature compared with the initial conditions before treatment (Figures 6B,

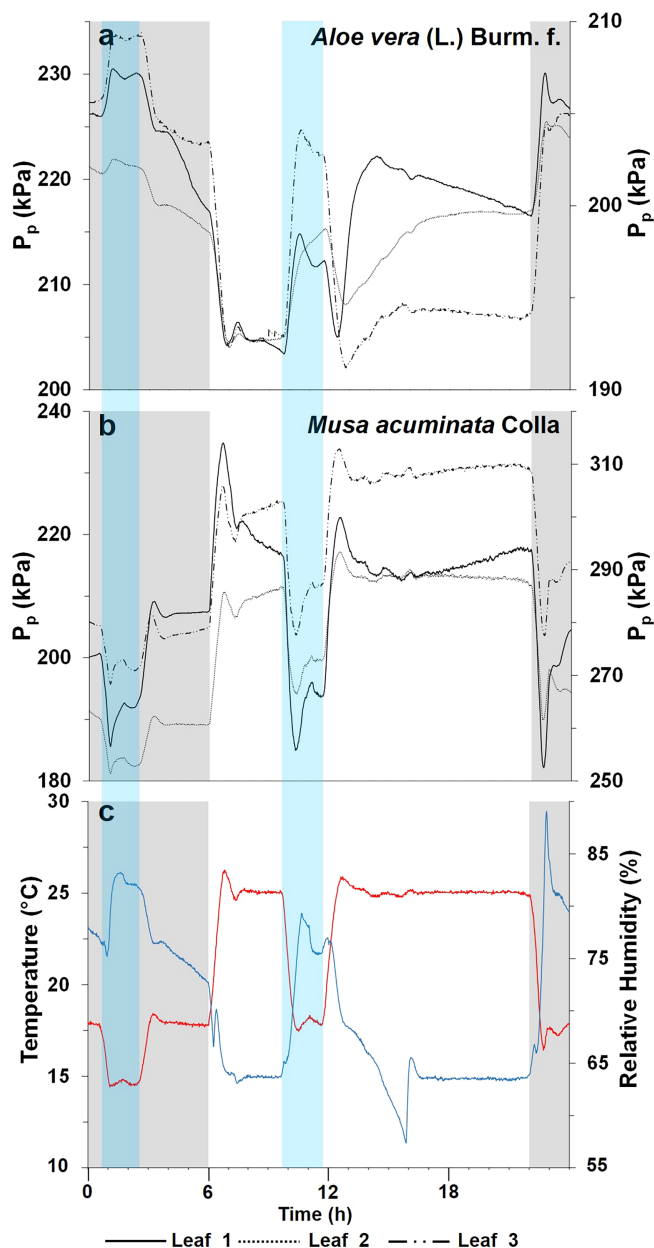


FIGURE 6 Effect of temperature on the LPCP output pressure, P_p , of (A) *Aloe vera* and (B) banana, under corresponding transient decreases in (C) temperature (red line) and increases in relative humidity (blue line) during the light and dark periods, as highlighted by the blue shaded areas. The P_p values of Leaf 1 are referenced to the right vertical axis. Dark period, as indicated in Figure 1.

C). The expected decrease in cell respiration, transpiration, and magnitude of the mesophyll osmotic potential due to a transient decrease in temperature and consequent increase in the relative humidity (Figure 6C), thereby decreasing VPD during the dark period all contribute to a transient decrease in the plant's water demand, thus increasing leaf turgor pressure. Consequently, the LPCP output pressure, P_p , of banana under these conditions will decrease as predicted by Equation (2) and observed experimentally (Figure 6B). Understandably, increasing the temperature and

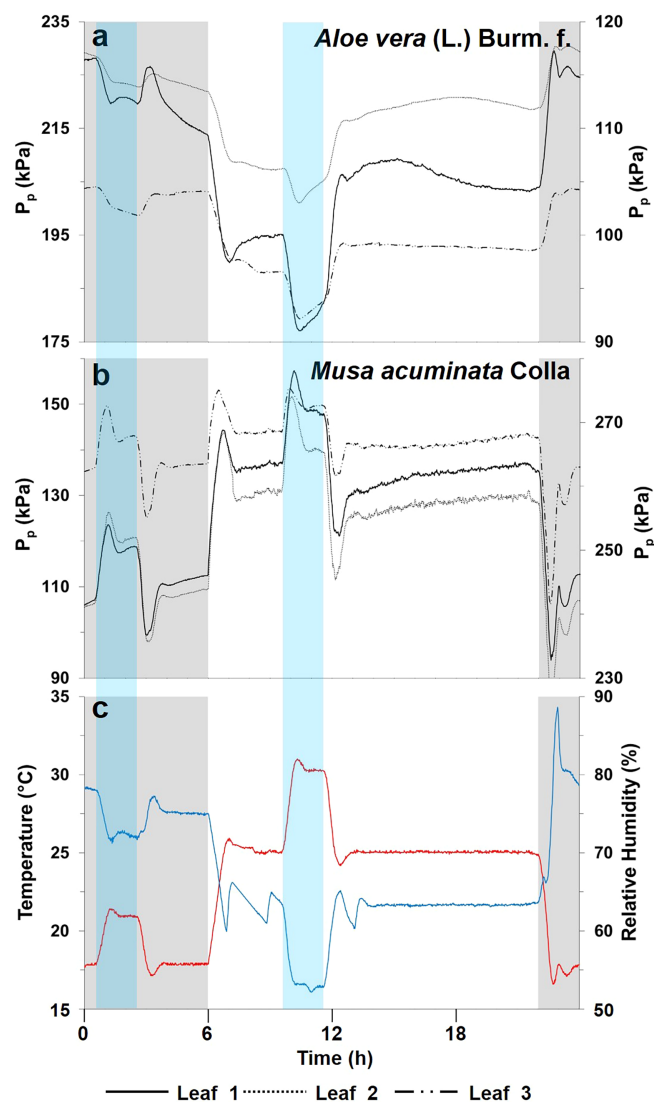


FIGURE 7 Effect of temperature on the LPCP output pressure, P_p , of (A) *Aloe vera* and (B) banana, under corresponding transient increases in (C) temperature (red line) and decreases in relative humidity (blue line) during the light and dark periods, as highlighted by the blue shaded areas. The P_p values of Leaf 1 are referenced to the right vertical axis. Dark period, as indicated in Figure 1.

decreasing the relative humidity from the normal conditions under which plants are grown (Figure 7C), both during the dark and the light periods, all these processes are reversed, as well as the plant's physiological responses (Figure 7B). Concomitant with an expected increase in transpiration (Figure 7C), caused by an increase in VPD, there is an increase in the output pressure, P_p , (Figure 7B), reflecting the decrease in leaf turgor pressure (Equation (2)) caused by the increase in water demand to sustain higher transpiration and photosynthetic activity, and decreased osmotic potential in banana during the light period. Also, higher water demand is expected to keep a higher cell respiration rate and transpiration, and lower osmotic potential under a transient higher temperature (Figure 7C) and lower VPD during the night period, leading to a decrease in the leaf turgor pressure that is

reflected by a concomitant increase in the banana LPCP output pressure, P_p , (Figure 7B).

As shown in Figures 6A and 7A, the response of *Aloe vera* output pressure, P_p , to transient changes in the ambient temperature and relative humidity (Figures 6C, 7C) are opposite to those observed on banana (Figures 6B, 7B), similarly to that observed on their normal diel patterns (Figures 1A, B). As noted above, such differences arise from their different photosynthetic pathways and associated leaf structure. By decreasing the ambient temperature, there is a simultaneous decrease in rubisco activity, leading to a transient increase in cytosolic CO_2 . Under such changing conditions, the activity of NAD-malic enzyme, also reduced by the decreasing temperature, is affected, favouring the build-up of malate in the cytosol to accommodate the increased CO_2 cytosolic levels. This, in turn, affects the exchange of malate between the cytosol and the vacuole, where it is stored, so that some may be retaken from the cytosol and stored back into the vacuole. Hence, the chlorenchyma osmotic potential is transiently lowered, overcoming the effect of decreasing temperature, and drawing more water from the hydrenchyma. The hydrenchyma turgor pressure decreases, consequently increasing the *Aloe vera* output pressure P_p (Figure 6A), contrary to that observed on banana (Figure 6B), as the temperature decreases (Figure 6C). A higher output pressure P_p is thus observed in *Aloe vera* (Figure 6A) once a lower temperature stabilizes compared to the ambient conditions before treatment (Figure 6C). Such scenario is also predicted by Equation (2), considering P_c as the hydrenchyma turgor pressure being sensed by the LPCP probes when applied on *Aloe vera* leaves. By lowering the temperature in the dark period (Figure 6C) and simultaneously decreasing VPD, the *Aloe vera* LPCP output pressure, P_p , is increased (Figure 6A), opposite to that observed on banana (Figure 6B). Such response can be seen as consequent of the decrease in the chlorenchyma osmotic potential due to an increased PEPC CO_2 -fixation and subsequent organic acid accumulation in the chlorenchyma. Such a decrease in the chlorenchyma osmotic potential draws more water from the hydrenchyma, leading to a decrease in its turgor pressure, i.e., an increase of LPCP output pressure, P_p (Equation (2), Figure 6B). Such response will be then reversed once ambient parameters return to pre-treatment levels (Figures 6A, C). As similarly observed on banana, once the ambient temperature is increased (Figure 7C), all these results are reversed, as shown by the decrease in the *Aloe vera* output pressure P_p (Figure 7A), indicating an increase in the hydrenchyma turgor pressure, as predicted by Equation (2). This stems from the decrease in drawing water from the hydrenchyma as the osmotic potential of the chlorenchyma increases to supply the increase in both the rubisco and the NAD-malic enzyme activities expected under higher photosynthetic rates and consequent CO_2 -demand. By increasing the temperature during the dark period (Figure 7C), there is a concomitant decrease in CO_2 -fixation by PEPC and organic acid accumulation in the chlorenchyma, increasing its osmotic potential and leading to a decrease in the water flow from the hydrenchyma, thus prompting to an increase in its turgor pressure, i. e., decrease in the output pressure, P_p , (Equation (2), Figure 7A). In both situations, the consequent changes in the accumulation of organic acids in chlorenchyma

resulting from the changes in PEPC CO_2 -fixation overcome the opposite effects of changing temperature on the chlorenchyma osmotic potential.

4 | DISCUSSION

Following the model first presented by Zimmerman et al. (2008) showing how the LPCP output pressure, P_p , relates to the leaf patch turgor pressure, P_c , of which banana is a good example (Cabrita, 2022; Zimmerman et al., 2010), and considering the leaf structure of *Aloe vera* plants (Chausser-Volfson and Gutterman, 2001; Silva et al., 2014) illustrated in Figure 8, the transfer of the pressure applied by the two magnets clamped to a *Aloe vera* leaf, P_{clamp} , throughout the leaf patch is such the that the measured LPCP output pressure, P_p , is given by:

$$P_p = \frac{K_1 K_2 K_3}{V_c V_h V_c} F_a P_{\text{clamp}} \quad (4)$$

where: K_1 , K_2 , and K_3 are constants; V_c and V_h are the leaf patch chlorenchyma and hydrenchyma volumes; and F_a is the attenuation factor, which on turgid plant tissues has been observed to be constant (Ehrenberger et al., 2012; Zimmermann et al. 2008, 2010). As the pressure applied by the magnets, P_{clamp} is also constant, Equation (4) above can be written as:

$$P_p = \frac{K}{\propto 2 V_h^3} P_{\text{in}} \quad (5)$$

where $K = K_1 K_2 K_3$ and the input pressure $P_{\text{in}} = F_a P_{\text{clamp}}$ are therefore constants, and.

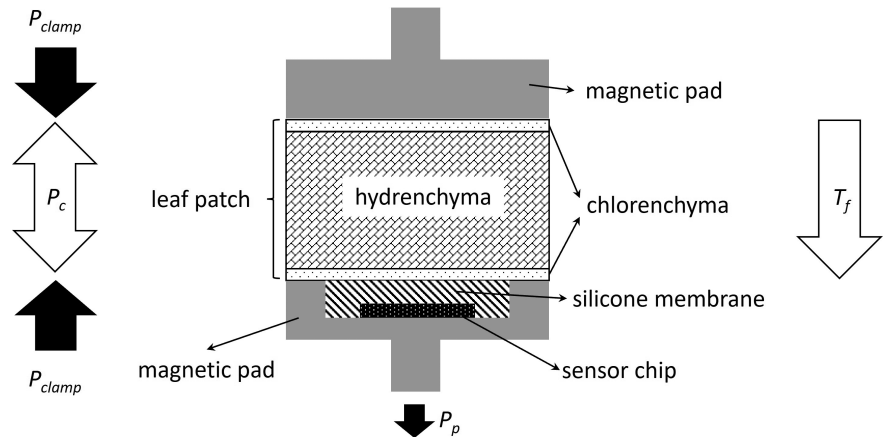
$$\propto = \frac{V_c}{V_h} \quad (6)$$

is the chlorenchyma to hydrenchyma volume ratio. As well known, plant cell volume varies with the turgor pressure, P_c , including CAM plants (Goldstein et al., 1991; Lüttge, 1986; Smith et al., 1987). Therefore, Equation (5) is a specific form of the more general Equation (2), considering the relationship between plant cell volume and turgor pressure, P_c , given by the definition of the volumetric elastic modulus, ε , (Broyer 1952; Philip 1958):

$$\varepsilon = V \left(\frac{\partial P_c}{\partial V} \right)_T \quad (7)$$

Under conditions that are far from inducing plasmolysis, the volumetric elastic modulus, $\varepsilon(P_c)$ (Equation (7)), can be described by a linear function (Ehrenberger et al., 2012; Murphy and Ortega, 1995; Steudle et al., 1977, Zimmermann et al. 2008). In these cases, Zimmerman et al. (2008) showed that the form of the function F in Equation (2) is an increasing power function of the leaf patch turgor

FIGURE 8 Leaf patch of *Aloe vera*: P_{clamp} – pressure applied by magnets; P_c – turgor pressure; P_p – leaf patch clamp probe (LPCP) output pressure; T_f – leaf patch transfer function.



pressure, P_c in which its dependence on P_c has been shown to be similar in many plant species (e. g., Ehrenberger et al., 2012; Martínez-Gimeno et al., 2017; Riboldi et al., 2016; Rüger et al., 2010a, b; Zimmerman et al., 2010, 2013). In *Aloe vera*, α has been observed to be as low as 0.16 and hardly changing under different soil water availability (Silva et al., 2014). Therefore, from Equation (5), the LPCP transfer function T_f (Cabrita, 2022; Westhoff et al., 2009; Zimmerman et al., 2008) that defines the value of the output pressure, P_p , in *Aloe vera* leaves (Figure 8) is given by:

$$T_f = \frac{K}{\alpha^2 V_h^3} \quad (8)$$

and is mostly determined by the leaf patch hydrenchyma volume, V_h (Figure 8). From Equation (5), one has that the change of the LPCP output pressure, P_p , with time in *Aloe vera* leaves is given by:

$$\frac{\partial P_p}{\partial t} = -P_p \left(\frac{3}{V_h} \frac{\partial V_h}{\partial t} + \frac{2}{\alpha} \frac{\partial \alpha}{\partial t} \right) \quad (9)$$

Using the definition of parameter α , Equation (9) above can be written as:

$$\frac{\partial P_p}{\partial t} = -P_p \left(\frac{1}{V_h} \frac{\partial V_h}{\partial t} + \frac{2}{V_c} \frac{\partial V_c}{\partial t} \right) \quad (10)$$

The hydraulic capacitance of the chlorenchyma, C_c , in species in which, similarly to *Aloe vera*, $\alpha < 0.3$, can be down to 2 orders of magnitude smaller than that of the hydrenchyma, C_h (Schulte et al., 1989; Smith et al., 1987). Considering the hydraulic capacitance as defined by Smith et al. (1987) and Equation (7), one can write:

$$C_i = \frac{V_i}{\epsilon_i}, \quad i = c, h \quad (11)$$

where subscripts c and h refer to chlorenchyma and hydrenchyma, respectively. For the diel pattern of water exchange between the chlorenchyma and the hydrenchyma and their associated volume

changes, Equation (11) implies that the chlorenchyma volumetric elastic modulus, ϵ_c , is bigger than that of the hydrenchyma, ϵ_h , as observed experimentally (Goldstein et al., 1991; Ogburn and Edwards, 2010). In species like *Aloe vera*, in which $\alpha < 0.3$, that difference can be up to one order of magnitude (Schulte et al., 1989; Smith et al., 1987). Hence, the smaller rigidity of the hydrenchyma cell wall, allowing it for greater changes in its volume with time, will attenuate any simultaneous opposite changes in the much smaller chlorenchyma volume, i.e., $\frac{1}{V_h} \left| \frac{\partial V_h}{\partial t} \right| \gg \frac{1}{V_c} \left| \frac{\partial V_c}{\partial t} \right|$. This means that the changes of the LPCP output pressure, P_p , with time, Equation (10), will follow those of the hydrenchyma essentially, even if attenuated by the smaller opposite changes in the chlorenchyma volume. These observations reflect the different roles and nature of both tissues in terrestrial CAM succulents, namely, the mechanical differences in the cell wall that allow the hydrenchyma to greatly change its volume and function as a tissue specialized in water storage specifically.

Considering previous results (Silva et al., 2014) and assuming that under the conditions described here, the chlorenchyma to hydrenchyma volume ratio, α , will remain basically unchanged, the changes in the volume of both the chlorenchyma and hydrenchyma during the diel CAM cycle in *Aloe vera*, although opposite, are smooth enough not to change α with time significantly. That is, one can assume the changes in parameter α with time negligible, i. e., $\frac{\partial \alpha}{\partial t} \approx 0$. Thus, in this scenario, Equation (9) can be written as:

$$\frac{\partial P_p}{\partial t} \approx -\frac{3P_p}{V_h} \frac{\partial V_h}{\partial t} \quad (12)$$

The changes in the values of the output pressure, P_p , in *Aloe vera* leaves are essentially dictated by changes in the hydrenchyma volume, V_h ; i.e., changes in its turgor pressure, P_c , ultimately. Thus, combining Equations (5), (7), and (8) and following the rationale to determine the dependence of the transfer function, T_f , Equation (8), on the hydrenchyma turgor pressure, P_c , (Cabrita, 2022; Westhoff et al., 2009; Zimmerman et al., 2008), one obtains that the LPCP output pressure, P_p , in *Aloe vera* leaves (Equation (5)) can be written as:

$$P_p = \left(\frac{b}{aP_c + b} \right)^{\frac{3}{\alpha}} P_{in} \quad (13)$$

where a and b are constants depending on the viscoelastic properties of the cell wall and the value and duration of the applied input pressure, P_{in} , (Westhoff et al., 2009; Zimmermann et al., 2008). From Equation (13), one has that the change in the LPCP output pressure, P_p , with time in *Aloe vera* leaves is given by:

$$\frac{\partial P_p}{\partial t} = -\frac{3P_p}{aP_c + b} \frac{\partial P_c}{\partial t} \quad (14)$$

Equation (13) and the consequent change of P_p with time (Equation (14)) are not that different from that first obtained by Zimmermann et al. (2008) and observed on many plant species, including banana (Cabrita, 2022; Zimmermann et al., 2010). The difference is that, in Equations (13) and (14), P_c is the hydrenchyma turgor pressure whereas, in species like banana, it is the overall mesophyll leaf patch turgor pressure that determines the LCPC output pressure, P_p , (Westhoff et al., 2009; Zimmermann et al., 2008). Using Equations (1) and (14), one obtains that the relaxation time constant in *Aloe vera* leaves Equation (1) can be written as:

$$\sigma_i = -\frac{3}{aP_c + b} \left(\frac{\partial P_c}{\partial t} \right)_i, i = \text{on, off} \quad (15)$$

which is slightly different from that obtained using the results of Zimmermann et al. (2008) that describe the LPCP output pressure, P_p , in several plant species, including banana:

$$\sigma_i = -\frac{1}{aP_c + b} \left(\frac{\partial P_c}{\partial t} \right)_i, i = \text{on, off} \quad (16)$$

Apart from the different origin of the turgor pressure being sensed by the leaf patch clamp pressure probes (LPCP), due to differences in the leaf structure of *Aloe vera* and banana and their associated values of parameters a and b , the different slopes of Equations (15) and (16), when considering $\frac{1}{aP_c + b} \left(\frac{\partial P_c}{\partial t} \right)_i$ as variables may also contribute to the differences observed in the magnitudes of the relaxation time constants in both species. That is more evident in the hypothetical scenario in which under the same input pressure, P_{in} , similar turgor pressure values are observed for similar values of parameters a and b . Considering the smaller value of the hydrenchyma volumetric elastic modulus, ϵ_h , higher values of the relaxation time constants would be then expected in *Aloe vera* leaves, reflecting its bigger changes in volume and turgor pressure when compared to those in banana. Such scenario is not totally implausible, considering the specialization of hydrenchyma as a water storage tissue with tightly packed cells (Chauser-Volfson and Gutterman, 2001; Nelson et al., 2005; Silva et al., 2014), which can change their volume greater and more rapidly than the banana mesophyll, composed of different cell types interspaced with humid air-filled intercellular spaces and no water storage specialization. A faster and more uniform distribution of

water would then be expected in the former rather than in the latter as a response to transient changes in VPD in the surrounding environment. The different magnitudes of the relaxation time constants of both species, although in the same order obtained from the results illustrated in Figure 1 as described above, do seem to agree with such hypothesis. However, the advantages of having a specialized tissue like the hydrenchyma seem most relevant under prolonged drought stress conditions as the internal distribution of water within *Aloe vera* leaves is seemingly unaffected by changes in the soil water availability and VPD, as suggested by the stability of the relaxation time constants (Figure 2). Oppositely, such internal processes in banana are highly affected by the amount of water available to plant roots (Figure 3). As time passes between irrigations, the balance between soil water availability, transpiration, and maintaining the plant's physiological and growth demands will be harder to keep as the internal water status is reduced. Under such conditions, at the end of each day that passes, the difference between the minimum and maximum leaf turgor pressure will increase, as reflected in the increasing values of ΔP_p (Figure 4B). Only the presence of a specialized water storage tissue composed of tightly packed cells could accommodate and buffer the effects of transient changes in the surrounding environment, even under adverse conditions. By occupying such a big fraction of the mesophyll volume, like in *Aloe vera*, it is therefore not surprising that changes in its volume, ultimately, turgor pressure, are that determining its diel P_p pattern (Figure 5B), following CAM photosynthesis (Figure 5A), unlike that observed on banana, a C_3 plant (Figures 1B, 4A).

Equation (13) explains the diel pattern of the LPCP output pressure, P_p , observed on *Aloe vera* leaves (Figures 1, 5), considering the well-known changes of the hydrenchyma volume with time during the diel photosynthetic cycle (Figure 5) observed on terrestrial CAM succulents (Eller and Ruess, 1986; Goldstein et al., 1991; Herrera et al., 2000; Howard and Nobel, 1986; Lüttge and Nobel, 1984; Ruess and Eller, 1985; Rygol et al., 1987; Schulte and Nobel, 1989; Schulte et al., 1989; Smith and Lüttge, 1985; Smith and Nobel, 1986; Smith et al., 1987; Steudle et al., 1975; Tissue et al., 1991). Specifically, the results here presented for *Aloe vera* (Figures 1, 5), as well as those predicted by Equation (13), strongly agree with the interaction between malate exchange in chlorenchyma cells and the changes in turgor pressure observed in the photosynthetic tissues of CAM plants (Lüttge et al., 1977; Smith et al., 1984). Namely, how the chlorenchyma water potential, highly affected by the use and exchange of malate between vacuoles and the cytosol during the CAM cycle, determines the changes in the hydrenchyma turgor pressure. Similarly, Equation (13) also explains the changes in P_p values observed on *Aloe vera* plants (Figures 6A, 7A) under transient changes in temperature, considering the changes in the hydrenchyma volume caused by the effects of temperature (Figures 6C, 7C) on CAM photosynthesis. The results presented by (Figures 6A, 7A) agree with the known effects of temperature on the chloroplast electron transport rate (Yamori et al., 2014) and rubisco activity (Crafts-Brandner and Salvucci, 2000) during CAM phase III. Such changes in the photosynthetic rate of CAM phase III, consequently affecting the chlorenchyma

water potential, are ultimately reflected in changes in the hydrenchyma volume, which are then sensed by LPCP probes (Figures 6A, 7A) and predicted by Equation (13). The results here described for banana (Figures 6B, 7B) have also been observed and explained by Cabrita (2022). Despite the effects of temperature on the photosynthetic rate of banana, i.e., rubisco activity, being similar to that of *Aloe vera*, as both species at the time of treatments perform the Calvin cycle, leaf turgor pressure is also affected by transpiration in banana while in *Aloe vera* there is practically no mass exchange with the surrounding environment, as most, if not all, stomata are kept shut at that time. The changes in *Aloe vera* P_p values to temperature changes during the dark period agree with the known effects of lower temperatures favouring nocturnal PEPC CO_2 -fixation in CAM plants (Buchanan-Bollig and Kluge, 1981; Carter et al., 1995; Lütge, 2006; Neales, 1973). The increase in nocturnal carboxylation in *Aloe vera* under lower temperatures, leading to a decrease in the chlorenchyma osmotic potential, increases the water flow from the hydrenchyma lowering its turgor pressure. Accordingly, that should thus increase the LPCP output pressure, P_p , in *Aloe vera* leaves as predicted by Equation (2) and observed experimentally (Figure 6B). Increasing the temperature during the dark period should cause the reverse response (Figure 7B) due to a decrease in the nocturnal carboxylation (PEPC) (Buchanan-Bollig and Kluge, 1981; Carter et al., 1995; Lütge, 2006; Neales, 1973).

Higher LPCP output pressure, P_p , values during the night period, in a similar pattern to that reported here for *Aloe vera*, were first reported on *Hippeastrum* plants (Cabrita, 2022). However, as Cabrita (2022) showed, the origin of the higher nocturnal P_p values in *Hippeastrum* plants is that by applying the LPCP probes on its leaves, one measures changes in the leaf aerenchyma air pressure and not leaf turgor pressure as in banana. Contrary to *Aloe vera* but similarly to banana, *Hippeastrum* are C_3 plants but with a different leaf structure that totally affects the transfer of pressure applied by the two magnets and the consequent measurement and interpretation of the output pressure P_p . Such differences can also be seen by comparing the relaxation time constants, which in *Hippeastrum* are about one order of magnitude lower than those observed on *Aloe vera* and banana. Such results highlight the importance of knowing in advance the leaf structure and physiology of the species on which one applies LPCP probes. The presence of differently specialized tissues, as well as the photosynthetic pathway, greatly affect the transfer of pressure through the leaf patch and consequently the diel output pressure, P_p , pattern. All species from the genus *Aloe* as well as those presenting a much higher proportion of hydrenchyma in their photosynthetic organs, e.g., those belonging to the genera *Agave*, *Sansevieria*, *Gasteria*, all very well-known examples of CAM plants, should present similar LPCP diel patterns to those observed on *Aloe vera*.

AUTHOR CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

ACKNOWLEDGEMENT

Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Paulo José Vieira Cabrita  <https://orcid.org/0000-0002-2620-3573>

REFERENCES

- Ball E., Hann J., Kluge M., Lee H.S.J., Lüttge U., Orthen B., Popp M., Schmitt A., Ting I.P. (1991) Ecophysiological compartment of the tropical CAM-tree *Clusia* in the field II. Modes of photosynthesis in trees and seedlings. *New Phytologist*, 117, 483–491.
- Barcikowski W., Nobel P.S. (1984) Water relations of cacti during desiccation: distribution of water in tissues. *Botanical Gazette*, 145, 110–115.
- Borland A.M., Leverett A., Hurtado-Castano N., Hu R., Yang X. (2018) Functional anatomical traits of the photosynthetic organs of plants with Crassulacean acid metabolism. In: W. Adams III, I. Terashima (Eds) *The Leaf: A Platform for Performing Photosynthesis. Advances in Photosynthesis and Respiration (Including Bioenergy and Related Processes)* 44. Springer, Cham, pp. 281–305.
- Broyer T.C. (1952) On volume enlargement and work expenditure by an osmotic system in plants. *Physiologia Plantarum*, 5, 459–469.
- Buchanan-Bollig I.C., Kluge M. (1981) Crassulacean acid metabolism (CAM) in *Kalanchoë daigremontiana*: Temperature response of phosphoenolpyruvate (PEP)-carboxylase in relation to allosteric effectors. *Planta*, 152, 181–188.
- Cabrita P. (2022) Non-invasive assessment of the physiological role of leaf aerenchyma in *Hippeastrum* Herb. and its relation to plant water status. *Planta*, 256: 19.
- Carter P.J., Wilkins M.B., Nimmo H.G., Fewson C.A. (1995) Effects of temperature on the activity of phosphoenolpyruvate carboxylase and on the control of CO_2 fixation in *Bryophyllum fedtschenkoi*. *Planta* 256, 375–380.
- Chausser-Volfson E., Gutterman Y. (2001) Anatomy, histochemistry and phytochemistry of leaves in *Aloe vera* var. *chinensis*. *Acta Botanica Sinica*, 43, 780–787.
- Crafts-Brandner S.J., Salvucci M.E. (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2 . *PNAS*, 97, 13430–13435.
- Davis S.C., Simpson J., Gil-Vega K.C., Niechayev N.A., van Tongerlo E., Castano N.H., Dever L.V., Búrquez A. (2019) Undervalued potential of Crassulacean acid metabolism for current and future agricultural production. *Journal of Experimental Botany*, 70, 6521–6537.
- Edwards E.J., Ogburn R.M. (2012) Angiosperm responses to a low CO_2 world: CAM and C_4 photosynthesis as parallel evolutionary trajectories. *International Journal of Plant Sciences*, 173, 724–733.
- Ehrenberger W., Rüger S., Rodríguez-Domínguez C.M., Díaz-Espejo A., Fernández J.E., Moreno J., Zimmermann D., Sukhorukov V.L., Zimmermann U. (2012) Leaf patch clamp pressure probe measurements on olive leaves in a nearly turgorless state. *Plant Biology*, 14, 666–674.
- Eller B.M., Ruess B.R. (1986) Modulation of CAM and water Balance of *Senecio medley-woodii* by environmental factors and age leaf. *Journal of Plant Physiology*, 125, 295–309.
- Goldstein G., Ortega, J.K.E., Nerd A., Nobel, P. (1991) Diel patterns of water potential components for the Crassulacean acid metabolism plant *Opuntia ficus-indica* when well-watered or droughted. *Plant Physiology* 95, 274–280.

- Herrera A. (2009) Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for? *Annals of Botany*, 103, 645–653.
- Herrera A., Fernández M.D., Taisma M.A. (2000) Effects of drought on CAM and water relations in plants of *Peperomia carnevalii*. *Annals of Botany*, 86, 511–517.
- Howard W.C., Nobel P.S. (1986) Nonsteady-state analysis of water flow and capacitance in *Agave deserti*. *Canadian Journal of Botany*, 64, 2556–2560.
- Kluge M., Knapp I., Kramer D., Schwerdtner I., Ritter H. (1979) Crassulacean acid metabolism (CAM) in leaves of *Aloe arborescens* Mill: comparative studies of the carbon metabolism of chlorenchyma and central hydrenchyma. *Planta*, 145, 357–363.
- Lambers H., Chapin III F.S., Pons T.L. (2008) *Plant Physiological Ecology*, Springer-Verlag New York, USA.
- Lee H.S.J., Lüttge U., Medina E., Smith J.A.C., Cram W.J., Diaz M., Griffiths H., Popp M., Schäffer C., Stimmel K.H., Thonke B. (1989) Ecophysiology of xerophytic and halophytic vegetation of a coastal alluvial plain in northern Venezuela III. *Bromelia humilis* Jacq., a terrestrial CAM bromeliad. *New Phytologist*, 111, 253–271.
- Leverett A., Hartzell S., Winter K., Garcia M., Aranda J., Virgo A., Smith A., Focht P., Rasmussen-Arda A., Willats W.G.T., Cowan-Turner D., Borland A.M. (2023) Dissecting succulence: Crassulacean acid metabolism and hydraulic capacitance are independent adaptations in *Clusia* leaves. *Plant, Cell & Environment*, 46, 1472–1488.
- Lüttge U. (1986) Nocturnal water storage in plants having Crassulacean acid metabolism. *Planta*, 168, 287–289.
- Lüttge U. (1988) Day-night changes of citric-acid levels in Crassulacean acid metabolism: phenomenon and ecophysiological significance. *Plant, Cell and Environment*, 11, 445–451.
- Lüttge U. (2004) Ecophysiology of Crassulacean acid metabolism (CAM). *Annals of Botany* 93: 629–652.
- Lüttge U. (2006) Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. *New Phytologist*, 171, 7–25.
- Lüttge U., Ball E., Greenway H. (1977) Effects of Water and Turgor Potential on Malate Efflux from Leaf Slices of *Kalanchoe daigremontiana*. *Plant Physiology*, 60, 521–523.
- Lüttge U., Nobel P. (1984) Day-night variations in malate concentration, osmotic pressure and hydrostatic pressure in *Cereus Validus*. *Plant Physiology*, 75, 804–807.
- Martínez-Gimeno M.A., Castiella M., Rüger S., Intrigliolo D.S., Ballester C. (2017) Evaluating the usefulness of continuous leaf turgor pressure measurements for the assessment of Persimmon tree water status. *Irrigation Science*, 35, 159–167.
- Murphy R., Ortega J.K.E. (1995) A New pressure probe method to determine the average volumetric elastic modulus of cells in plant tissues. *Plant Physiology*, 107, 995–1005.
- Neales T.F. (1973) The effect of night temperature on CO₂, assimilation, transpiration, and water use efficiency in *Agave Americana* L. *Australian Journal of Biological Sciences*, 26, 705–714.
- Nelson E.A., Sage T.L., Sage R.F. (2005) Functional leaf anatomy of plants with Crassulacean acid metabolism. *Functional Plant Biology*, 32, 409–419.
- Nobel, P.S. (1976) Water relations and photosynthesis of a desert CAM plant *Agave deserti*. *Plant Physiology*, 58, 576–582.
- Nobel, P.S. (1977) Water relations and photosynthesis of a barrel cactus, *Eurocactus acanthodes*, in the Colorado desert. *Oecologia*, 27, 117–133.
- Nobel, P.S. (1983) *Biophysical Plant Physiology and Ecology*, W H Freeman & Co, San Francisco, USA.
- Ogburn R.M., Edwards E.J. (2010) The Ecological water-use strategies of succulent plants. In J.C. Kader, M. Delseny (Eds.) *Advances in Botanical Research* 55, Academic Cambridge, USA, pp. 179–255.
- Osmond C.B. (1978) Crassulacean acid metabolism: a curiosity in context. *Annual Review of Plant Physiology*, 29, 379–414.
- Philip J.R. (1958) The osmotic cell, solute diffusibility, and the plant water economy. *Plant Physiology*, 33, 264–271.
- Riboldi L.B., Oliveira R.F., Angelocci L.R. (2016) Leaf turgor pressure in maize plants under water stress. *Australian Journal of Crop Science*, 10, 878–886.
- Ruess B.R., Eller B.M. (1985) The correlation between Crassulacean acid metabolism and water uptake in *Senecio medley-woodii*. *Planta*, 166, 57–66.
- Rygel J., Büchner K.H., Winter K., Zimmermann U. (1986) Day/night variations in turgor pressure in individual cells of *Mesembryanthemum crystallinum* L. *Oecologia*, 69, 171–175.
- Rygel J., Winter K., Zimmermann U. (1987) The relationship between turgor pressure and titratable acidity in mesophyll cells of intact leaves of a Crassulacean-acid-metabolism plant, *Kalanchoë daigremontiana* Hamet et Perr. *Planta*, 172, 487–493.
- Rüger S., Ehrenberger W., Arend M., Geßner P., Zimmermann G., Zimmermann D., Bentrup F.-W., Nadler A., Raveh E., Sukhorukov V.L., Zimmermann U. (2010a) Comparative monitoring of temporal and spatial changes in tree water status using the non-invasive leaf patch clamp pressure probe and the pressure bomb. *Agricultural Water Management*, 98, 283–290.
- Rüger S., Netzer Y., Westhoff M., Zimmermann D., Reuss R., Ovadiya S., Gessner P., Zimmermann G., Schwartz A., Zimmermann U. (2010b) Remote monitoring of leaf turgor pressure of grapevines subjected to different irrigation treatments using the leaf patch clamp pressure probe. *Australian Journal of Grape and Wine Research*, 16, 405–412.
- Schmidt J.E., Kaiser W.M. (1987) Response of the succulent leaves of *Peperomia magnoliaefolia* to dehydration. *Plant Physiology*, 83, 190–194.
- Schulte P.J., Nobel P.S. (1989) Responses of a CAM plant to drought and rainfall: capacitance and osmotic pressure influences on water movement. *Journal of Experimental Botany*, 210, 61–70.
- Schulte P.J., Smith J.A.C., Nobel, P.S. (1989) Water storage and osmotic pressure influences on the water relations of a dicotyledonous desert succulent, *Plant, Cell and Environment*, 12, 831–842.
- Silva H., Sagardia S., Ortiz M., Franck N., Opazo M., Quiroz M., Baginsky C., Tapia C. (2014) Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability. *Revista Chilena de de Historia Natural*, 87, 13.
- Smith J.A.C., Lüttge U. (1985) Day-night changes in leaf water relations associated with the rhythm of Crassulacean acid metabolism in *Kalanchoë daigremontiana*. *Planta*, 163, 272–282.
- Smith J.A.C., Nobel P.S. (1986) Water movement and storage in a desert succulent: anatomy and rehydration kinetics for leaves of *Agave deserti*. *Journal of Experimental Botany*, 37, 1044–1053.
- Smith J.A.C., Schulte P.J., Nobel, P.S. (1987) Water flow and water storage in *Agave deserti*: osmotic implications of Crassulacean acid metabolism. *Plant, Cell and Environment*, 10, 639–648.
- Smith, J.A.C., Steudle E., Lüttge U. (1984) Day-night changes in cell turgor pressure during crassulacean acid metabolism (CAM) measured with the pressure probe. In: W.J. Cram, K. Janáček, R. Rybová, K. Sigler (Eds.) *Membrane Transport in Plants*. Academia, Czechoslovak Academy of Sciences, Praha, pp. 119–120.
- Steudle E., Lüttge U., Zimmermann U. (1975) Water Relations of the Epidermal Bladder Cells of the Halophytic Species *Mesembryanthemum crystallinum*: Direct Measurements of Hydrostatic Pressure and Hydraulic Conductivity. *Planta*, 126, 229–246.
- Steudle E., Zimmermann U., Lüttge U. (1977) Effect of Turgor Pressure and Cell Size on the Wall Elasticity of Plant Cells. *Plant Physiology*, 59, 285–289.
- Tissue D.T., Yakir D., Nobel P. (1991) Diel water movement between parenchyma and chlorenchyma of two desert CAM plants under dry and wet conditions. *Plant, Cell and Environment*, 14, 407–413.
- Westhoff M., Reuss R., Zimmermann D., Netzer Y., Gessner A., Geßner P., Zimmermann G., Wegner L.H., Bamberg E., Schwartz A., Zimmermann U.

- (2009) A non-invasive probe for online-monitoring of turgor pressure changes under field conditions. *Plant Biology*, 11, 701–712.
- Winter K., Garcia M., Virgo A., Smith J.A.C. (2021) Low-level CAM photosynthesis in a succulent-leaved member of the Urticaceae, *Pilea peperomioides*. *Functional Plant Biology*, 48, 683–690.
- Yamori W., Hikosaka K., Way D.A. (2014) Temperature response of photosynthesis in C₃, C₄, and CAM plants: temperature acclimation and temperature adaptation. *Photosynthesis Research*, 119, 101–117.
- Zimmermann D., Reuss R., Westhoff M., Geßner P., Bauer W., Bamberg E., Bentrup F.-W., Zimmermann U. (2008) A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. *Journal of Experimental Botany*, 59, 3157–3167.
- Zimmermann U., Rüger S., Shapira O., Westhoff M., Wegner L.H., Reuss R., Gessner P., Zimmermann G., Israeli Y., Zhou A., Schwartz A., Bamberg E., Zimmermann D. (2010) Effects of environmental parameters and irrigation on the turgor pressure of banana plants measured using the non-invasive, online monitoring leaf patch clamp pressure probe. *Plant Biology*, 12, 424–436.
- Zimmermann U., Bitter R., Marchiori P.E.R., Rüger S., Ehrenberger W., Sukhorukov V.L., Schüttler A., Ribeiro R.V. (2013) A non-invasive plant-based probe for continuous monitoring of water stress in real time: a new tool for irrigation scheduling and deeper insight into drought and salinity stress physiology. *Theoretical and Experimental Plant Physiology*, 25, 2–11.
- Zotz G., Hietz P. (2001) The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany*, 52, 2067–2078.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cabrita, P.J.V. (2024) Water exchange between the Chlorenchyma and the Hydrenchyma and its physiological role in leaves with Crassulacean acid metabolism. *Physiologia Plantarum*, 176(2), e14221. Available from: <https://doi.org/10.1111/ppl.14221>