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Physiological characteristics of the Atlantic Forest native bromeliads: *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker

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ABSTRACT. Despite the ecological importance of bromeliads, the basic knowledge about the physiological aspects in some species is poorly understood. The aim of this study was to physiologically characterize the species *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker to contribute to a better understanding of their metabolic processes. From mature leaves of bromeliads *N. campo-alegrense* and *A. ornata* held at the Agricultural Sciences Center of the Federal University of Santa Catarina, it was quantified photosynthetic pigments, carbohydrates and starch, and determined stomata and trichome density. The chlorophyll and carotenoids content did not differ significantly between species. However, the total carbohydrates content of *A. ornata* (3.2 mg g^{-1} FM) was significantly higher than *N. campo-alegrense*, but with similar starch content (1.7 mg g^{-1} FM). Both species present hypostomatic leaves, with more trichomes in the species *A. ornata*. This information provides the groundwork for future studies on the biochemical mechanisms related to their photosynthetic process, contributing in establishing strategies for the conservation and propagation of these species.

Keywords: Bromeliaceae, carbohydrates, carotenoids, chlorophyll.

Características fisiológicas de bromélias nativas da Mata Atlântica: *Nidularium campo-alegrense* Leme e *Aechmea ornata* Baker

RESUMO. Apesar da importância ecológica das bromélias, o conhecimento básico sobre os aspectos fisiológicos em algumas espécies é pouco compreendido. O objetivo do presente trabalho foi caracterizar fisiologicamente as espécies *Nidularium campo-alegrense* Leme e *Aechmea ornata* Baker, para contribuir ao melhor entendimento dos seus processos metabólicos. Pelas folhas maduras, das bromélias *N. campo-alegrense* e *A. ornata* mantidas no Centro de Ciências Agrárias (CCA) da Universidade Federal de Santa Catarina (UFSC), foram quantificados os pigmentos fotossintéticos, carboidratos e amido e foi determinada a densidade estomática e tricomática. Os teores de clorofila e carotenoides não apresentaram diferença significativa entre as espécies. No entanto, na espécie *A. ornata*, o teor de carboidratos totais (3,2 mg g^{-1} MF) foi significativamente maior e o teor de amido (1,7 mg g^{-1} MF) semelhante quando comparado com *N. campo-alegrense*. As duas espécies apresentam folhas hipoestomáticas e o número de tricomas foi maior na espécie *A. ornata*. Estas informações fornecem as bases para futuros estudos sobre os mecanismos bioquímicos relacionados com seu processo fotossintético para contribuir no estabelecimento de estratégias de conservação ou propagação destas espécies.

Palavras-chave: Bromeliaceae, carboidratos, carotenoides, clorofila.

Introduction

Bromeliaceae is one family belonging to the order Poales (APG, 2009) and has 3,170 species in 60 genera (LUTHER, 2008). Except for *Pitcairnia feliciana* (A. Chev.) Harms and Mildbr., which occurs in Africa, the other species of the family are distributed throughout the tropical and subtropical zone of the Americas, highlighting South America as the center of great diversity (BENZING, 2000). Besides its ecological importance, characteristics and colors of the leaves and flowers are of great commercial value in worldwide (GUERRA; DAL VESCO, 2010). And concerning the latter plants have been illegally extracted from their natural habitats, increasing the risk of future extinction (COFFANI-NUNES, 2002).

Representatives of the Bromeliaceae family may be found under different conditions of altitude, temperature and humidity (REITZ, 1983), and can be terrestrial, epiphytic or rupicolous (SMITH; DOWNS, 1974). Approximately half of all bromeliads are epiphytes, and plants successfully grow under such conditions due to their capability to develop strategies to intercept, absorb and store rainwater efficiently (BENZING, 2000). In these conditions their representatives show ecophysiological, morphological and anatomical adaptations (LORENZO et al., 2010). According to Gutschick (1999), abiotic and biotic factors affect leaves structural characteristics by changing the stomatal opening mechanism and stomatal density and distribution, leaf area, trichomes and defensive chemical compounds. Besides these, a large number of epiphytic bromeliad show the Crassulacean acid metabolism (CAM); a photosynthetic pathway which minimizes gas exchange. In these case, the stomata in the leaves remain shut during the day to reduce evapotranspiration, but open at night to collect carbon dioxide (LUTTGE, 2008). According to Luttge (2010), CAM metabolism is an effective strategy for both plant acclimatization and adaptation, and also to increase plant diversity and survival under stress conditions.

In the Bromelioideae subfamily are included 29 genera and about 760 species (BENZING, 2000), concentrated mainly in the Atlantic Forest (MARTINELLI et al., 2008). Among the representatives of this subfamily, occurring in the southeastern and southern Brazil, are endemic species of *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker (FORZZA et al., 2013), in which the aspects related to the physiology of metabolism are poorly known. Studies that generate this type of basic knowledge allow further identification of some specific point that could contribute to its conservation.

The aim of this study was to physiologically characterize the bromeliads *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker, as for the quantification of starch, carbohydrates, carotenoids and chlorophyll content, and density of trichomes and stomata; important physiological parameters in the analysis of plant metabolism.

Material and methods

Vegetable material

Plants of *N. campo-alegrense* and *A. ornata* belong to the bromeliad at the Center of Agricultural Sciences (CCA) of the Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina State, Brazil. The plants were kept under plastic screens providing 50% shading. In this environment, the photosynthetic photon flux density (PPFD) near the leaves of *N. campo-alegrense* was 195 μmol m^{-2} s^{-1} and for *A. ornata* it was 128 μmol m^{-2} s^{-1}. The average temperature during the collection period was 18.3ºC.

For the analysis, segments were collected in the middle part of healthy leaves of four specimens of each species.

Total chlorophyll and carotenoids content

One gram of fresh matter was macerated in liquid N, using ceramic crucible. Then, 30 mL of acetone (85%) was added and subjected to stirring for two minutes. Subsequently, the solution was filtered using a suction pump and the volume adjusted to 50 mL with acetone (85%). The absorbances of the resulting solutions were measured in a spectrophotometer at 470, 646 and 663 nm. The contents of chlorophylls and carotenoids of tissues were expressed in μg of the pigment per gram of fresh matter (μg g^{-1} FM), being estimated from equations 1, 2 and 3, proposed by Lichtenthaler and Wellburn (1983):

$$\text{Chlorophyll a} = 12.21\ (A663) - 2.81\ (A646) \quad (1)$$

$$\text{Chlorophyll b} = 20.13\ (A646) - 5.03\ (A663) \quad (2)$$

$$\text{Carotenoids} = (1000A470 - 3.27\ [\text{chl a}] - 104[\text{chl b}])/227 \quad (3)$$

In addition to that, the total chlorophyll was calculated (Chlorophyll a + Chlorophyll b) and the a/b chlorophyll ratio.

Total soluble carbohydrates content

One gram of fresh matter was macerated using liquid N. Then, the material was placed in falcon tubes and 5 mL of ethanol (80%) were added and kept in boiling water (100°C) for 5 minutes Then, the extracts were centrifuged at 3000 rpm for 10 minutes The supernatant was filtered and the volume adjusted to 10 mL with ethanol (80%). Were removed 50 μL from the extract and then there was the addition of 0.5 mL of distilled water, 0.5 mL of phenol (5%) and 2.5 mL of concentrated sulfuric acid (96%) (DUBOIS et al., 1956). The absorbance was measured at 490 nm. The total carbohydrates content was estimated from a standard curve ($y = 0.018x – 0.037$; $r^2 = 0.996$), and expressed in milligrams of glucose per gram of fresh matter (mg g^{-1} FM).

Starch content

An addition of 10 mL of distilled water at 4°C and 13 mL of perchloric acid (52%) was made to the precipitate resulting from the extraction of carbohydrates, being stirred for 15 minutes. Then, there was an addition of 20 mL of distilled water and the solution was centrifuged at 3,000 rpm for 15 minutes. To the residue, there was an addition of 5 mL of ice-cold distilled water (4°C) and 6.5 mL of perchloric acid (52%), being stirred for 15 minutes. The solution was centrifuged at 3,000 rpm for 15 minutes. The supernatant was decanted into a beaker for uniting the fractions of starch. The solution was homogenized and filtered through glass wool and the initial 5 mL were discarded. The volume was adjusted to 10 mL using ethanol (80%). Were removed 50 μL from the extract and 0.5 mL of distilled water were added, 0.5 mL of phenol (5%) and 2.5 mL of concentrated sulfuric acid (96%) (DUBOIS et al., 1956). The absorbance was measured at 490 nm. The total carbohydrates content was estimated from a standard curve ($y = 0.005x - 0.014$; $r^2 = 0.995$), and expressed in milligrams of glucose per gram of fresh matter (mg g^{-1} FM).

Density of stomata and trichomes

In the central part of the plants leafs, paradermic sections in adaxial and abaxial surfaces were performed manually, using razor blades. The sections were placed on slides, with Toluidine Blue and, subsequently, analyzed by optical microscopy (Olympus® BX-40) for determining the distribution and quantification of the leaf stomata and trichomes. Sections were photographed using a camera (Olympus DP71) coupled to the microscope.

Statistical analysis

The contents of chlorophyll, carotenoids, carbohydrates and starch, as well as count data of trichomes and stomata, were subjected to analysis of variance (ANOVA) and, when significant, the means were subjected to the SNK test of mean comparison, at a 5% error level. All analyzes were performed in the statistical software SISVAR (FERREIRA, 2011).

Results

Chlorophyll and total carotenoids content: Although *A. ornata* showed the highest values in the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids; no significant difference was found between the two pigments of the species assessed. However, the ratio of chlorophyll a/b for the species *N. campo-alegrense* (3.07) was similar to the species *A. ornata* (2.94) (Table 1).

Table 1. Chlorophyll and carotenoids content (μg g^{-1} FM) in the leaf tissue of *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker.

Bromeliad	Chlorophyll a	Chlorophyll b	Chlorophyll total	Chlorophyll a/b Ratio	Carotenoids
	----------μg g^{-1}FM-----------				μg g^{-1} FM
Nidularium campoalegrense Leme	56.4±11.2a	18.3±4.2a	74.7±15.2a	3.07	14.3±1.8a
Aechmea ornata Baker	97.1±11.2a	33.0±4.2a	130.2±15.2a	2.94	17.7±1.8a

Means (± SE) in a vertical column followed by the same letter do not differ significantly according to SNK test at a level of 5%. FM= Fresh matter.

Total soluble carbohydrates and starch content: The total soluble carbohydrates content found in *N. campo-alegrense* (2.10 mg g^{-1} FM) was significantly lower than that obtained in *A. ornata* (3.2 mg g^{-1} FM). While the starch content in the middle part of the leaf in *N. campo-alegrense* (2.57 mg g^{-1} FM) and *A. ornata* (1.7 mg g^{-1} FM) showed no significant differences (Figure 1).

Density of stomata and trichomes: The leaves of the two bromeliads evaluated are hypostomatic. In *N. campoalegrense*, the stomatal density was 36 stomata per mm^2 and in *A. ornata* was 9 stomata per mm^2 (Figure 2). For both species, were found peltate trichomes distributed in longitudinal rows on both surfaces of the leaf (Figure 3). *A. ornata* showed an average of 13.4 and 14.3 per mm^2 in the adaxial and abaxial surface respectively. The number of trichomes in *N. campo-alegrense* was smaller at approximately 9 and 4 trichomes per mm^2 in the adaxial and abaxial surface respectively (Figure 2).

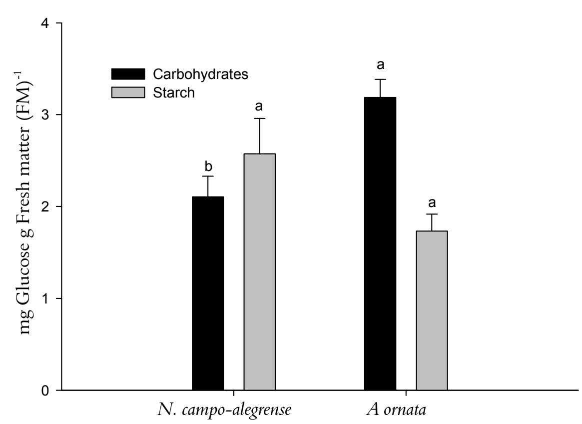

Figure 1. Carbohydrate and starch content (mg g^{-1} FM) in the leaf of *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker. Vertical bars represent the standard error. FM= Fresh matter.

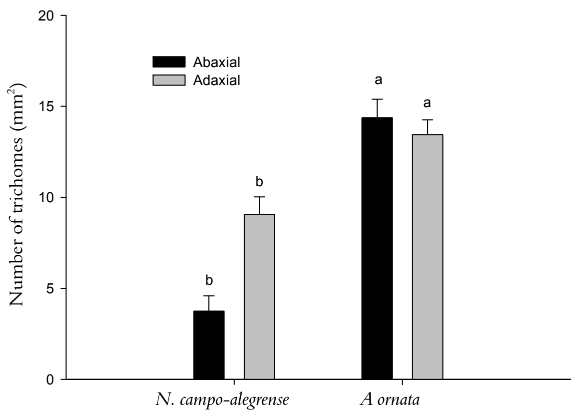

Figure 2. Average number of trichomes on the abaxial and adaxial surface of the leaf tissue of *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker. Vertical bars represent the standard error.

Discussion

The chlorophyll is highly sensitive to reduction in brightness (ENGEL; POGGIANI, 1991), and the chlorophyll and carotenoids tend to increase their concentration, with reduced light intensity (LIMA et al., 2006). According to Reinbothe et al. (2010) the chlorophyll absorbs sunlight and participates in energy transfer during photosynthesis. On the other hand, carotenoids play a role in photoprotection, preserving the chlorophylls from the oxidative destruction of O_2 (DA SILVA MESSIAH et al., 2001). Thus, the relationship between the concentration of total chlorophyll and carotenoid concentration is important for protecting against photo-oxidation, especially in leaf senescence (RIGON et al., 2012).

The chlorophyll contents can also be used as an estimate of the total content of nitrogen and consequently the nutritional status of the plant (SALEEM et al., 2010; AGUERA et al., 2010). The results obtained with bromeliads by Kurita et al. (2012), show the relationship between photosynthetic pigments content and nitrogen content in the environment.

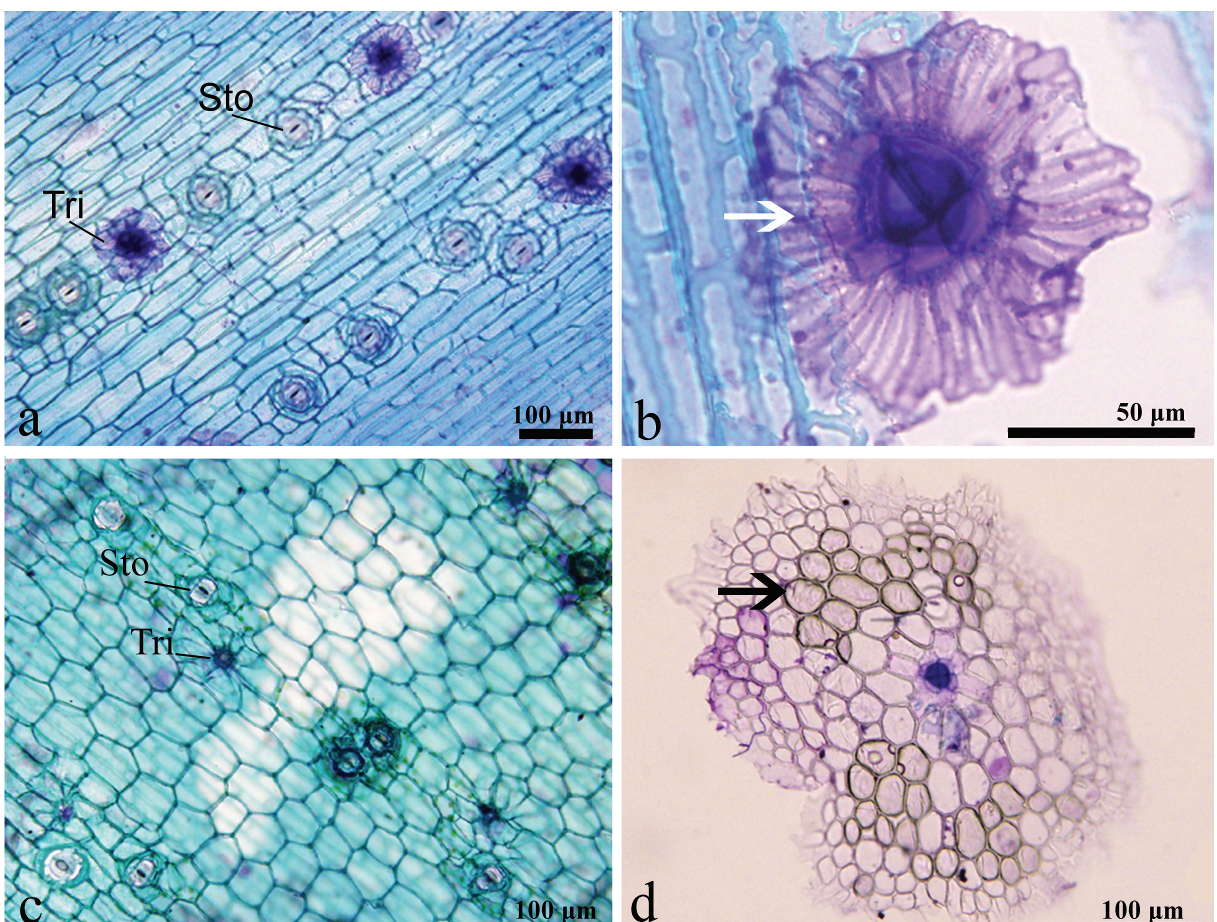

Figure 3. Front view of the epidermis of leaves of *Nidularium campo-alegrense* Leme and *Aechmea ornata* Baker. Distribution of stomata and trichomes in: a) *N. campo-alegrense*; c) *A. ornata*. Detail of trichomes in: b) *N. campo-alegrense* showing elongated cells (white arrow); d) *A. ornata* showing rounded cells (black arrow). Tri: Trichomes; Sto: stomata.

The ratio of chlorophyll a/b can indicate the composition of the chloroplast (BRITO et al., 2011) and according to Nakazono et al. (2001) this ratio is increasing the greater the amount of light. However, the epiphytic bromeliad *Guzmania monostachia* L. Rusby ex Mez var. *monostachia*, the ratio of chlorophyll a/b remains constant at low and high irradiation conditions (MAXWELL et al., 1999). This was also observed in several epiphytic species of Bromeliaceae, in which there were no statistically significant differences in the ratio of chlorophyll a/b acclimated to high and low solar irradiance (GRIFFITHS; MAXWELL, 1999). According to Dai et al. (2009), the chloroplastid pigments are important factors related to photosynthetic efficiency in plants, affecting their growth and their adaptability to environments with different levels of luminosity. However, the content of photosynthetic pigments (chlorophyll and carotenoids) found in *A. ornata* and *N. campo-alegrense* was similar to contents found by Gonçalves et al. (2011) in greenhouse-grown bromeliads.

Likely, the higher carbohydrate content in *A. ornata* is related to the higher content of chlorophyll found in this species. The higher the chlorophyll content, greater the proportion of incident light absorbed (BJORKMAN, 1981). And this can be associated to a higher photosynthetic rate and, consequently, an increase in carbohydrates production. According to Majerowicz (2008), through the flow of solar energy, channeled through photosynthesis, compounds with low energy are converted into energy-rich compounds, such as carbohydrates, mainly sucrose and starch, which are more stable products of the photosynthetic process. This carbon is exported to several parts of the plant to support the growth of various organs (PENG et al., 2011). Therefore, sugars availability is related to plant development (GIBSON, 2004).

In other species of ornamental bromeliads, the total soluble carbohydrates and starch content were lower compared to this study (UARROTA et al., 2012). This may be related to the characteristics of each species, the location of the tissue collected for analysis and the environmental conditions. In the apical portion of the leaves of the bromeliad *Vriesea gigantea* it was found the highest concentrations of starch. This suggests that this region has the highest photosynthesis rates in relation to the base, and consequently the higher availability of energy, reducing power and carbon skeletons (TAKAHASHI et al., 2007). The greatest accumulation of sugars in the portion of the leaf apex was also observed in *Ananas comosus* cv. *Spanish Red* (POPP et al., 2003).

The hypostomatic leaves found in both species assessed correspond to findings in other species of the subfamily Bromelioideae (MONTEIRO et al., 2011; PEREIRA et al., 2011). According to Derwiduee and Gonzalez (2010) it is characteristic of the species Bromelioideae to have hypostomatic leaves with a wavy abaxial surface, forming troughs where stomata are located and trichomes are inserted. The position of the stomata seems to be a characteristic more related to the phylogeny of the group than to the environmental conditions (SCATENA; SEGECIN, 2005). According to Proença and Sajo (2004), stoma restricted to the abaxial leaf blade is a likely plesiomorphic feature in the bromeliad family.

According to Pimenta (2004), the density of stomata is an interesting parameter, because the stomata is the structure responsible for the regulation of gas exchange, directly influencing photosynthesis and productivity. In Bromeliaceae, the increase in stomatal density on sun leaves, compared to the shadow leaves, was observed in *A. bromeliifolia* (SCARANO et al., 2002). This increase in stomatal density in response to increased availability of light, implies higher transpiration rates, contributing to the cooling of the leaves (MARKESTEIJN et al., 2007; ROZENDAAL et al., 2006). This temperature reduction is central to the realization of the biochemical mechanisms of photosynthesis (DEMMIG-ADAMS; ADAMS, 1996). However, Volenikova and Ticha (2001), found that the nutritional status of the plant affect stomatal density more than light irradiation.

Trichomes are associated with absorption of water and nutrients directly from the atmosphere, reducing perspiration and the effects of sunstroke, protection from predators and pathogens, and attraction of pollinators and dispersers (BENZING, 2000). According to Derwiduee and Gonzalez (2010), this character is important because the scales provide outstanding coverage of the epidermal surface, finding the genus *Tillandsia* bromeliads trichomes that completely cover the stomata. However, the leaves of bromeliads evaluated in this study showed trichomes distributed in longitudinal rows which do not completely cover the epidermis, presenting exposed stomata (Figure 3). The morphology and density of trichomes range interspecific, and also within an individual, depending upon the location on the leaf and plant age (ADAMS; MARTIN, 1986; REINERT; MEIRELLES, 1993). Derwiduee and Gonzalez (2010) found in species of the genus *Aechmea*, trichomes with the peltate portion formed by cells with little differentiation extending radially and firmly adhering to the epidermis, making it difficult to distinguish the contour of the scale. These characteristics are clearly seen in *A. ornata* presenting rounded cells in the peltate portion of trichomes (Figure 3). However, in *N. campo-alegrense* cells in this region are elongated. According to Tardivo and Cervi (1997), the genus *Nidularium* presents leaf scales characterized by a small group of cells in the central area and radial elongated cells. In the family Bromeliaceae, the subfamily Bromelioideae presents the greatest differences in shapes of scales (PEREIRA et al., 2011). The intensity of solar radiation, water deficiency and different environmental factors can lead to changes in area and leaf thickness, stomatal and trichome density (GUTSCHICK, 1999).

Conclusion

The bromeliads *N. campo-alegrense* and *A. ornata,* exhibit physiological characteristics associated with their natural habitat, with strategies to prevent photo-oxidative damage caused by exposure to high light intensity to maximize water saving and for absorbing water and nutrients. Both species showed similar results regarding levels of photosynthetic pigments and starch. However, they presented significant differences in the content of total soluble carbohydrates. Both have hypostomatic leaves, but density of stomata and trichomes differed between species. Information on the physiological aspects of these species is important to know their metabolism and provide the foundation for future studies related with the photosynthetic process, as well as being used for the establishment of conservation or propagation strategies.

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