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Mucilage secretion in the inflorescences of *Aechmea blanchetiana*: Evidence of new functions of scales in Bromeliaceae

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ABSTRACT

The peltate trichomes of Bromeliaceae comprise an important feature of this plant group. Although recognized for their capacity for water and nutrient absorption, little is known concerning their secretory functions. This study aimed to identify and describe the structures observed to secrete mucilage in the inflorescences of *Aechmea blanchetiana,* a species of Bromeliaceae endemic to Brazil. Samples of different portions and developmental stages of inflorescences were collected and processed for study under light and electron microscopy. Mucilage is secreted by peltate trichomes covering the inflorescence axis, which have a structure typical of bromeliad scales, although often having an irregular shield commonly exhibiting a stellate outline. Secretion is performed by cells comprising the so-called wing portion of the trichome and begins even in initial stages of inflorescence expansion. In expanded portions, secretory activity ceases and the remaining secretion appears as a thin dehydrated film covering the inflorescence surface. Ultrastructural data show the presence of well-developed Golgi apparatus and endoplasmic reticulum, confirming a secretory function related to non-cellulosic polysaccharide production. We believe that the mucilage is related to protection against desiccation, especially at early stages of expansion. Such a functional role, in association with the nature and dynamics of the secretion, suggests that these structures act as colleters. As far as we know, this is the first record for exogenous secretory activity by the typical peltate trichomes of Bromeliaceae, and the first time when colleter-like functions have been considered and discussed in this family.

1. Introduction

The peltate trichomes of Bromeliceae, often referred as scales, are an important feature of the family and much effort has been made to elucidate their structure, function and adaptative significance (e.g., Benzing, 1970, 1976, 2000; Benzing and Burt, 1970; Brighigna et al., 1988; Dolzmann, 1964, 1965; Sakai and Sanford, 1980; Smith and Downs, 1974, 1977, 1979; Stefano et al., 2008; Tomlinson, 1969; Varadarajan and Gilmartin, 1987). Different functions have been attributed to these structures, such as protection against transpiration and excess radiation, pollinator and disperser attraction and defense against herbivores and pathogens, among others (Benzing, 2000). Despite many of the studies regarding the functional roles of bromeliad having been related to their capacity for water and nutrient absorption (Benzing, 1970, 1976; Benzing et al., 1976; Benzing and Burt, 1970; Brighigna et al., 1988; Ohrui et al., 2007), very little has been done to investigate their secretory activity in this taxon.

In fact, Benzing (2000) reported that secretory activity by bromeliad trichomes occurs in only two species of the genus *Brocchinia*, in *Navia glandulifera* B.Holst and in *Ronnbergia petersii* L.B.Sm. (synonym of *Aechmea allenii* L.B.Sm.). However, the secretory function in *Brocchinia* is potentially related to the strategy of carnivory attributed to a few species of the genus (Givnish et al., 1984), and the secretory activity in these cases needs further confirmation. Also, in *Navia glandulifera* and *Ronnbergia petersii* secretory capacity seems to only have been inferred based on the presence of capitate trichomes, even though there have been no detailed investigations of the possible secretions or the structure of these trichomes. In a broad survey of bromeliad anatomy, Tomlinson (1969) also adressed secretion in Bromeliaceae, devoting special attention to gummosis and mucilaginous secretions, emphasizing that “the tendency for Bromeliaceae to develop mucilaginous substances requires investigation”. Nonetheless, very little information has since been acquired regarding mucilage secretion by bromeliads. Accumulation of hydrophilic material in the distal peduncular cells of foliar trichomes was reported for some *Tillandsia* species and *Ananas comosus* (L.) Merr. (Brighigna et al., 1988; Dolzmann, 1964; Papini et al., 2010; Sakai and Sanford, 1980), while the presence of such substances in the trichomes was interpreted by Brighigna et al. (1988)

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as evidence of an intracellular secretion, suggesting a glandular function related to water absorption efficiency. Papini et al. (2010) also discussed the glandular function in trichomes of *Tillandsia*, and stated that the presence of such substances inside the trichomes is "obviously necessary for water uptake". Exogenous secretion of mucilaginous substances was briefly reported by Benzing (2000) for some bromeliads species, but only clearly demonstrated in *Alcantarea mucilaginosa* Leme (Leme, 2009) and *Vriesea bituminosa* Wawra (Monteiro and Macedo, 2014). In all of the above cases, however, there have been no further investigation into secretory activity, and it remains unknown whether secretion is carried out by peltate trichomes or not.

Specifically, the presence of glandular structures associated with the protection of meristematic and young portions of the plant body (i.e., colleters), although very common in eudicotyledons (Thomas, 1991), remains poorly sampled among monocotyledons and other groups (Cardoso-Gustavson et al., 2014; Leitão and Cortelazzo, 2008; Mayer et al., 2011; Oliveira et al., 2017). In Bromeliaceae, the presence of colleters has been verified only in association with the vegetative axis of *Aechmea blanchetiana* (Ballego-Campos, *unpublished results*), and despite the important ecophysiological role that these structures may have, very little is known regarding their presence in Bromeliaceae.

The tank-bromeliad *Aechmea blanchetiana* is a terrestrial bromeliad endemic to northeast and southeast Brazil, where it occurs mostly in the sandy coastal zones ("Restingas") of the Brazilian Atlantic forest (Forzza et al., 2015; Kanashiro et al., 2007; Smith and Downs, 1979). Due to its ornamental value, the species is reported to be predatorily explored, which puts it under threat in its natural sites of occurrence (Kanashiro et al., 2007; Santa-Rosa et al., 2013). Within the "Restingas", bromeliads and other plants are known to play an important role in the intricate dynamics of vegetation succession, especially by acting as "nurse plants" (Scarano, 2002) and therefore providing suitable conditions for plant establishment. However, while doing so, plants in such ecosystems have to cope with a harsh environment, which imposes a set of adverse conditions such as high salinity, oligotrophy, high temperatures, strong winds and drought (Scarano, 2002; Scarano et al., 2001).

By increasing the capacity of water retention and avoiding desiccation, colleters and mucilage secretion act indirectly in the water economy and in the protection of young organs, what must be especially important in dry and stressful ecosystems such as those inhabit by *A. blanchetiana*. In addition, considering that many aspects of the water economy in Bromeliaceae (e.g. absorbing trichomes, water impoundment tanks, CAM photosynthesis) have been understood as important factors for the evolution and diversification of the family (Givnish et al., 2014), the role of mucilaginous secretion must be addressed.

Thus, in the present study we aimed to identify and describe the secretory structures involved in the production of the mucilage observed in the inflorescences of *Aechmea blanchetiana* and to discuss the potential ecological roles of this secretory system.

2. Material and methods

2.1. Sampling and plant material

Inflorescences of adult specimens of *Aechmea blanchetiana* were collected at both early and late stages of development. Inflorescences at an early stage were considered those which axis were completely covered with bracts and showed no apparent lateral ramification. Inflorescences showing strong ramification and exposed axis were considered those in late stages of development. Sampling was conducted in two population of *Aechmea blanchetiana* growing on the campus of Universidade Federal de Minas Gerais (Minas Gerais State, Brazil), both cultivated in open gardens fully exposed to sunlight. A minimum of five individuals was sampled from each population, and voucher specimens were deposited in the BHCB herbarium of the Universidade Federal de Minas Gerais.

2.2. Light microscopy

For anatomical investigation, samples of lateral axes, bracts and floral parts were collected from inflorescences at both above mentioned stages of development. Several degrees of expansion were sampled, allowing analysis throughout the fully development of inflorescences. Fragments of the sampled material were then fixed in Karnovsky fixative (pH 7.2 in 0.1 M phosphate buffer; modified from Karnovsky, 1965) under moderate vacuum, dehydrated in an ethanol series and embedded in (2-hidroxiethyl)-methacrylate (Historesin embedding kit, Leica TM, Heidelberg, Germany). Transverse and longitudinal sections (6 μm thick) were obtained with a rotary microtome, stained with toluidine blue (pH 4.7; modified from O'Brien et al., 1964), counterstained with ruthenium red (0.002% aqueous solution) and mounted with Entellan®. Histochemical tests were conducted by staining sections using ruthenium red alone (Johansen, 1940) and Sudan Red B (Brundrett et al., 1991) for detection of acidic polysaccharides and lipophilic substances, respectively.

Macroscopic analyses were also conducted with the aid of a stereomicroscope (Zeiss, Stemi 2000-C; Canon Power Shot A650).

2.3. Electron microscopy

For scanning electron microscopy (SEM), samples of lateral axes and bracts at both early and late stages of expansion (parts not exposed, with less than 50% of expansion; and parts exposed and fully expanded, respectively) were fixed using Karnovsky fixative (pH 7.2 in 0.1 M phosphate buffer; modified from Karnovsky, 1965), dehydrated in an ethanol series, critical-point dried using CO_2, gold coated and examined using a scanning electron microscope (Quanta 200; FEI Company, Eindhoven, Netherlands). Some of the samples were washed under distilled water prior to fixation in order to remove excess mucilage.

For transmission electron microscopy (TEM), sampling was restricted to portions of lateral axes at the early stage of expansion (less than 50% of expansion). Samples were vacuum fixed in Karnovsky fixative (pH 7.2 in 0.1 M phosphate buffer; modified from Karnovsky, 1965), post-fixed using 1% osmium tetroxide (pH 7.2 in 0.1 M phosphate buffer), dehydrated in an ethanol series and embedded in epoxy resin (Spurr, 1969). Ultrathin sections were obtained and examined using a transmission electron microscope (Tecnai G2-Spirit; Philips/FEI Company, Eindhoven, Netherlands) at 80 kV.

3. Results

3.1. Distribution and secretory activity

The inflorescences of *A. blanchetiana* displayed a central axis that branches heavily. Inflorescences at early stages of development were entirely covered by pale green bracts and did not show visible lateral branches. As they expand, the central and lateral axes are gradually exposed to the environment and displayed changes in coloration, becoming remarkably red and yellow (Fig. 1A). Trichomes (scales) were present on the abaxial face of sepals and bracts, along the entire axis and on the outer ovarian wall.

In young inflorescences, a large amount of secretion was present covering the surface of bracts and axes, often collecting along the small spaces present in the compact internode system that comprises the inflorescence at this stage. Under such conditions, the secretion was abundant, hyaline (Fig. 1B) and somewhat viscous. Tests using ruthenium red indicated the presence of acidic polysaccharides in this exudate, which will henceforth be considered as mucilage.

Although mucilage was present covering all distinct parts of the inflorescence, we did not observe signs of its exudation by the epidermis or any other tissue of the developing bracts, sepals or outer ovarian wall. In these portions, the cells that comprise the trichomes did not show indications of secretory activity, and were usually