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Research article

Trichome-like emergences in *Croton* of Brazilian highland rock outcrops: Evidences for atmospheric water uptake

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ABSTRACT

Background and aims: Although trichomes are typical features of the species-rich genus *Croton*, little is known about their development, anatomical structure and function. This study aims to characterize the anatomy of leaf trichomes of three *Croton* species restricted to Brazilian rock outcrops, and investigate their functional role in an environment with limited soil water availability.
Methods: Samples of leaves at different stages of maturity were submitted to standard anatomical light and scanning electron microscopy techniques to perform a structural and developmental study. Atmospheric water uptake was investigated through the absorption of aqueous solutions of berberine hemisulfate and Indian ink.
Key results: The so-called trichomes actually correspond to emergences because they originate from protodermal and ground meristem activity rather than from epidermal tissue. These emergences show a complex anatomy, are closely associated with the mesophyll sclereids and might be involved in leaf atmospheric water uptake. The outermost cells of the emergences are arranged radially and present non-lignified thick walls. We demonstrate that an aqueous solution can effectively penetrate through the leaf emergences, continuing through sclereids and reaching the vascular bundle or flowing across the mesophyll maximizing water distribution in the leaf tissue.
Conclusions: The complex structure and hypothesized function of *Croton* trichome-like emergences represent a novel finding in Euphorbiaceae. In the three species studied, these leaf emergences might play a role in atmospheric water absorption and they may be one of the clues for the occurrence of *Croton* in habitats with limited soil water supply where this genus is usually species-rich and abundant.

1. Introduction

Trichomes of diverse types characterize *Croton* L. (Euphorbiaceae), one of the largest genera of flowering plants (Berry et al., 2005) with over 1200 species (Govaerts et al., 2000). Because of their great diversity in morphology, density, and distribution patterns on the plant, trichomes have been used by *Croton* systematists as a rich source of characters at different taxonomic levels (Webster, 1993; Lima and Pirani, 2003, 2008; Riina et al., 2010; Caruzo et al., 2011; van Ee and Berry, 2011; Vitarelli et al., 2015).

Plant trichomes are specialized epidermal structures originating from protodermal cells. The existence of a connection between trichomes and structures in the mesophyll has been reported in several species of *Croton* (Solereder, 1908; Metcalfe and Chalk, 1950; Sá-Haiad, 1987; Starling, 1993; Louro et al., 2003; Appezzato-da-Glória and Carmello-Guerreiro, 2006; Lucena and Sales, 2006; Silva, 2006; Sá-Haiad et al., 2009; Barros and Soares, 2013), but without any experimental demonstration about the trichomes functional role.

In *Croton*, seven types of trichomes (stellate, fasciculate, multiradiate-rosulate, dendritic, lepidote, papillate, and glandular) have been described (Webster et al., 1996). Trichomes are important features in systematics, physiology and ecology, however studies about their anatomy and development are scarce. We

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set to conduct a detailed study of leaf trichomes of three closely related species of *Croton* section *Lamprocroton*, namely *Croton erythroxyloides* Baill., *C. pygmaeus* L.R. Lima, and *C. splendidus* Mart. ex Colla. In a recent taxonomic study, lepidote trichomes were reported for the abaxial side of the leaves of *C. pygmaeus* and *C. splendidus* and on both leaf surfaces in *C. erythroxyloides*, and stellate trichomes on the adaxial side of the leaves of *C. pygmaeus* and *C. splendidus* (Lima and Pirani, 2008).

Croton erythroxyloides, *C. pygmaeus* and *C. splendidus* are common in high elevation rocky habitats in southeastern Brazil; the so called *campos rupestres* and *campos de altitude* (Lima and Pirani, 2008). Plants living in these ecosystems are exposed to similar extreme environmental conditions, such as high daily variation of temperature, risk of frost, intensive sun irradiation, strong winds, and the nutrient-impoverished shallow soils with a low water-holding capacity that leads to long periods of severe water limitation for plant productivity (e.g., Safford, 1999; Benites et al., 2003; Lüttge et al., 1998; Morales et al., 2015; Silveira et al., 2015). These conditions exert continuous selective pressure on plants colonizing these environments, which could result in the evolution of morphoanatomical and/or physiological adaptations (Eames and McDaniels, 1947; Metcalfe and Chalk, 1983; Hamba et al., 2002; Alcantara et al., 2015), especially those involved in water economy. Many species have superficial root systems that would allow for rapid use of rain pulses (Nobel et al., 1990). Whilst leaves of resurrection plants are physiologically inactive during the dry season, differing from deciduous species in that the former retain leaf nutrients during the dry season (Griffiths et al., 2014). Aerial roots with velamen or pseudostems are common in Velloziaceae (Porembski and Barthlott, 1995), Bromeliaceae (Pita and Menezes, 2002) and Orchidaceae (Silva et al., 2010). Velamen may play a role in accessing moisture from dew and rain in *campos rupestres* and *campos de altitude* (Moreira et al., 2009; Silva et al., 2010) or could be more important in preventing water loss than in moisture or nutrient uptake (Dycus and Knudson, 1957).

Among the anatomical traits, trichomes stand out by their widespread presence in phylogenetically unrelated species (Araújo et al., 2010; Lusa et al., 2014; Francino et al., 2015). They might have different functions, such as protection against herbivores either by physical (Levin, 1973; Wagner, 1991) or chemical defense (Levin, 1973), reduction of the fraction of radiation that is absorbed by leaves (Fahn, 1986; Karabourniotis and Esseas, 1996), protection of stomata and reduction of water loss by evapotranspiration (Fahn, 1986). Trichomes may play a role in atmospheric water uptake, performing as specialized epidermal structures that could speed the atmospheric water absorption process (Stuart, 1968; Benzing et al., 1976; Martin and von Willert, 2000; Fernández et al., 2014). Besides trichomes, other epidermal structures, such as hydathodes and cuticule structure, could be related to atmospheric water absorption (Grundell, 1933; Meidner, 1954; Leyton and Armitage, 1968; Riederer 2006; Eller et al., 2013). All of these epidermal structures seem to provide an important alternative water supply, especially in environments where soil water availability is deficient. Several experiments could be conducted to evaluate the water pathway through epidermal structures, for example, using berberine sulfate fluorochrome (Heide-Jørgensen, 1990), aqueous safranin solution (Milanez and Machado, 2008), and measuring the hydric potential in wettable leaves (Fernández et al., 2014).

This study aims to perform anatomical, histochemical and ontogenetic characterization of leaf trichomes of three closely related *Croton* species occurring in similar habitats, i.e., *campos rupestres* and *campos de altitude*, as well as to evaluate their functional role in plants growing in such extreme and harsh environments characterized by limited soil water availability.

2. Materials and methods

2.1. Tissue sampling and study site

Croton erythroxyloides, *C. pygmaeus* and *C. splendidus* were chosen because they present different types of trichomes (lepidote, stellate, and fasciculate) and occur in extreme habitats, allowing us to compare trichome structure, development, and function under similar environmental conditions. Leaf samples were obtained from natural populations or from herbarium material (Table 1). Voucher specimens were deposited in the herbarium of the Universidade Federal de Viçosa (VIC).

Croton erythroxyloides and *C. splendidus* were collected from natural populations (Fig. 1A–E) in *campos de altitude* above 1700 m elevation on 'Serra das cabeças', 'Parque Estadual da Serra do Brigadeiro' (PESB), Minas Gerais State, Brazil (Fig. 1A–C). *Campos de altitude* (altitudinal grasslands) are rupestrian ecosystems of igneous formation that occur mostly on the 'Serra da Mantiqueira' and 'Serra do Mar' ranges in southeastern Brazil. *Campos rupestres* and *campos de altitude* share several plant genera and are known to harbor considerable numbers of endemic species (Safford, 2007; Alves and Kolbek, 2010). In spite of their floristic and physionomic similarities, they are geologically distinct (Benites et al., 2007; Alves and Kolbek, 2010). For example, *campos de altitude* have more floristic affinities with the páramo vegetation of the Andes than *campos rupestres* (Alves and Kolbek, 2010). On the other hand, both ecosystems have soils developed on ironstones that are shallow and acidic with a low water-holding capacity.

The PESB is located at the summit of 'Serra da Mantiqueira' between 42°20′ and 42°40′S and 20°20′ and 21°00′W (Engevix, 1995). The regional climate is classified as mesothermal medium – CWb (Koeppen, 1948), the average annual precipitation is 1300 mm. Although, the annual precipitation does not imply a xeric environment, the high sun light exposition, shallow depth and high permeability of soils and intense wind, produce an environment with extreme low water supply (Benites, 1998). The rainy season is from November to March and the dry season from May to September. Leaf samples were collected in both the dry and rainy season. The average annual temperature is 18 °C (64.4 °F) (Engevix, 1995), with June, July and August as the coldest months. The recorded minimum temperature was below 0 °C. The presence of clouds covering the peaks of the PESB is observed daily, especially in early morning hours (Fig. 1B and C). The individuals sampled grow in dense grass tussocks directly on bare rock outcrops with low water-holding capacity (Fig. 1C).

Samples of *Croton pygmaeus* were obtained from herbarium material (Table 1). We were unable to obtain fresh material from this species because of the difficult and expensive logistics due to its disjunct location from the other two species. According to the herbarium collection labels the individuals sampled are shrubs 1–2 m tall, growing directly on rocky substrates in the state of Rio Grande do Sul, southern Brazil (approximate location at 30°32′6.92″S, 53°33′2.14W).

Leaves at different developmental stages (leaf primordia, young and mature leaves) from each specimen were used in this study. Four specimens of *Croton splendidus*, four of *C. erythroxyloides* and two of *C. pygmaeus* were used as replicates (Table 1), using a minimum of three leaves per specimen. Sections of leaf samples were taken from the apical, median and basal portions of the blade (including midrib, margin, and region between them).

2.2. Light microscopy

Leaf tissue sampled from herbarium specimens was boiled in distilled water for 10 min, treated with 2% potassium hydroxide for 2 h at room temperature, rinsed in tap water five times, dehy-