Morphoanatomy of the underground system of *Androtrichum trigynum* (Cyperaceae)

*Morfoanatomia do sistema subterrâneo de Androtrichum trigynum (Cyperaceae)*

Roberta Andressa Pereira¹ & Ana Claudia Rodrigues²,³

Abstract

*Androtrichum* has only one species, *A. trigynum* (Spr.) Pfeiffer, occurring in coastal regions of the southwestern Atlantic coast. It presents an underground system consisting of rhizomes and adventitious roots. The rhizome is thickened, plagiotropic, sympodial, and floral scapes and roots arise from it. From the stem promeristem, the protoderm, procambium and ground meristem are differentiated. At the apex region, the intercalary meristem and primary thickening meristem (PTM) are observed. The adventitious roots originate from the PTM, and present root apex with closed organization. The young epidermis has papillose cells, and meristematic endoderm activity is observed. In a mature root, the outer cortex, or hypodermis, and the internal cortex can be identified. The endoderm presents radially elongated cells with thin walls and the pericycle is biseriate. The anatomical features observed in the present study are found in other species of Cyperaceae and some xeromorphic characters can be identified.

Key-words: xeromorphic features, adventitious roots, rhizomes.

Introduction

*Androtrichum trigynum* (Spr.) Pfeiffer is the only species of the genus *Androtrichum*, Cyperaceae (Alves et al. 2009). Popularly known as junco-da-praia (Cordazzo et al. 2006), its geographic distribution is restricted to the southwest Atlantic coast, occurring from Argentina to southern Brazil (Costa et al. 1988). It is considered to be a heath, halophyte plant, abundant on the dunes settled in dry and humid areas. According to Cordazzo et al. (2006) swamps and depressions, periodically flooded, constitute habitats of great complexity and biological diversity of the coastal dunes, and *A. trigynum* has great importance in ecological structuring of these habitats due to its dominance in these areas.

Metcalfe (1971) describes, in general, the anatomy of the vegetative organs of many Cyperaceae species, as well as Kukkonen (1967); Eiten (1969);
when the spaces are formed following the lysis of some cortical cells, or by both processes, as observed in *Cyperus giganteus* (Rodrigues & Estelita 2004).

On the other hand, the structural adaptations that may occur in such organs by the action of the environment cannot be ignored (Fahn & Cutler 1992). The coastal dunes (restingas) present a highly stressful environment, causing species to develop a number of morphological, anatomical, physiological and reproductive adaptations (Cordazzo et al. 2006). Thus, this study aims to describe the underground system of *Androtrichum trigynum* (Spr.) Pfeiffer, focusing on the principal phases of differentiation of the tissues and highlighting possible morphoanatomical adaptations to the environment.

**Materials and Methods**

Specimens of *Androtrichum trigynum* (Spr.) Pfeiffer (Cyperaceae) were collected from the restinga of Parque Municipal das Dunas da Lagoa da Conceição, Florianópolis, located on the eastern coast of Santa Catarina Island (Florianópolis, SC), between the coordinates 27º38'20,7''S; 48º27'69,1''W.

According to Strahler’s classification, the city of Florianópolis (SC) has a subtropical humid climate, categorized as sub-hot (CECCA 1996). According to Beltrame et al. (2006), the temperatures of the studied area vary between 15ºC and 18ºC in winter, and between 24ºC and 30ºC in summer. Precipitation is well distributed during the whole year (average rate of 1,521mm) (CECCA 1996), and relative humidity of the air has an annual average rate of 82% (Herrmann 1989).

Part of the collected material was deposited in the “FLOR” herbarium under the number 37,276. Samples of roots and rhizomes, at different stages of development, were fixed in glutaraldehyde 2.5% in 0.1M, pH 7.2 sodium phosphate buffer (Feder & O’Brien 1968) for 24 hours. Posteriorly, they were washed in the same buffer and preserved in ethanol 70%. Permanent slides were made with material embedded in paraffin and hydroxyethylmethacrylate resin. For paraffin, apical parts of the material were dehydrated in increasing tertiary butanol series (Johansen 1940), pre-infiltrated in paraffin oil and butanol, infiltrated in pure paraffin in three stages in an incubator at 60ºC. For resin,
the manufacturer’s instructions were followed. The material immersed using both techniques was sectioned with a steel razor using a Leica RM2125 rotary microtome, 7 to 10 µm thick. The sections in hystoresin were stained with toluidine blue (O’Brien et al. 1965), and the sections in paraffin were stained with astra blue and safranin (Bukatsch 1972).

Semi-permanent slides were made from handmade sections of the mature material, with the aid of a proper razor; stained with safranin and astra blue (Bukatsch 1972) and mounted with glycerinated gelatin (Kraus & Arduin 1997). Histochemical tests were carried out on fixed material, using Lugol for starch (Johansen 1940), Sudan III for lipophilic substances (Sass 1951), ferric chloride for phenolic substances, acidified phloroglucinol for lignin and ruthenium red for mucilage (Johansen 1940). The photomicrographs were made using a Leica MPS 30 DMLS microscope with a built-in digital camera.

**Results**

**Morphology**

*Androtrichum trigynum* is aphyllous, with a perennial and photosynthetic floral scape. The underground system consists of rhizomes and adventitious roots (Fig. 1a). The rhizome is thickened, plagiotropic and sympodial, with small internodes, and covered by reddish-brown cataphylls; from the axils, side buds emerge. From the rhizome, arise the floral scapes whose base is also covered by reddish-brown cataphylls. Roots arise from the rhizome internodes. When young, they have a light coloration; when mature, a dark-brown coloration.

Anatomy – rhizome

In a longitudinal section from the stem apex, the promeristem can be observed surrounded by early cataphylls (Fig. 1b). The apical meristem has a tunic-body formation, the tunic consisting of two to three cell layers (Fig. 1c), and the body, of many cell layers. From the promeristem, the protoderm, the procambium and the fundamental meristem are distinguished (Fig. 1b). The intercalary meristem can also be seen at the apical region (Fig. 1b) consisting of, approximately, 15 cell layers with thin walls and arranged in a stratified way (Fig. 1d) and the formation of side buds of the same composition as the apical bud (Fig. 2a).

From approximately 720 µm from the apex, the primary thickening meristem (PTM) starts its activity. It has a circular shape, delimiting the cortical region and the vascular cylinder (Fig. 2b), producing centripetally, through periclinal divisions, vascular strands and parenchymatous cells, and only parenchymatous cells centrifugally (Fig. 2c). The PTM also originates adventitious roots (Fig. 2d). The epidermis is uniseriate, and the cortex, initially, is homogeneous (Fig. 2d).

Following the development of the organ, the activity of the PTM gradually diminishes, and its endoderm and pericycle are distinguished (Fig. 3a-b). The endoderm presents radially elongated cells with thin walls (Fig. 3b). The pericycle is uniseriate, with isodiametric cells and thickened walls (Fig. 3b). At the vascular cylinder, there are isodiametric, conspicuous parenchymatous cells, with thin walls and few intercellular spaces (Fig. 3c). All of the vascular bundles are amphyvasal and present a sheath of cells with thickened walls (Fig. 3c-d). The outer bundles are formed by the PTM (Fig. 2c, 3a-b), and the inner bundles are formed by the procambium (Fig. 3c-d).

At maturity, the inner cortex and the outer cortex are distinguished (Fig. 4a). The outer cortex, or hypodermis, consists of compact hexagonal parenchymatous cells with thin walls (Fig. 4a). The inner cortex is formed by smaller cells in relation to the external cortical cells. They are isodiametric and with small intercellular spaces (Fig. 4a). It can also be observed that the innermost cells of this cortex, including the endoderm cells, have thickened walls (Fig. 4b-c). Many idioblasts having phenolic compounds are found throughout the whole rhizome (Fig. 2d, 3c, 4b-d), and in a lesser amount at the outer cortex (Fig. 4a). Phenolic compounds can also be observed on the walls of the endoderm cells and of the innermost cortical cells (Fig. 4c-d). Starch grains occur at the inner cortex (Fig. 4a), as well as at the vascular cylinder (Fig. 4b).

Anatomy – roots

The adventitious roots arise from the rhizome starting from the PTM (Fig. 2d). At the root apex, the root cap originates from periclinal divisions of the calyptron, which consists of a group of cells with conspicuous nuclei, arranged in compressed layers. The promeristem, the protoderm, the procambium and the fundamental meristem are also observed (Fig. 5a-c).
From the promeristem, the initial cell common to the protoderm and the meristem is observed (Fig. 5b). The cylinder of procambium is easily observed, the promeristem region being very small (Fig. 5a-b). The protoderm cells gradually elongate radially as they drift away from the initial cell (Fig. 5c).

In transverse sections 120 μm from the root apex, the cylinder of procambium, the beginning of meristematic activity of the endoderm and the root cap, still present, with many cell layers, is observed (Fig. 5d). The meristematic endoderm forms, through periclinal divisions, the radiated cortical region (Fig. 5d-e).
On the sections 280 μm from the root apex (Fig. 6a), we can observe 3 of 4 layers of root cap cells and the epidermis differentiated in papillose cells. Those have dense content and secrete a thick layer of substance, which is deposited between those cells and the root cap cells (Fig. 6b). The cortical region, at this stage of development, consists of outer cortex or hypodermis and inner cortex. The hypodermis (Fig. 6a, 6c) originates from the fundamental meristem and presents approximately six layers of isodiametric cells, with no intercellular spaces. The inner cortex (Fig. 6a, 6d) results from periclinal divisions of the meristematic endoderm, showing about 22 cell layers radially disposed, with thin walls and conspicuous intercellular spaces (Fig. 6d). At the vascular cylinder, the vascular elements are in differentiation (Fig. 6a, 6d).

From 1610 μm from the apex (Fig. 6e-g), the root cap cells disappear, and a considerable layer of substance secreted by the epidermal cells can still be observed, which present dense content as well as the outermost cells of the hypodermis (Fig. 6e-f). The meristematic endoderm ceases
its activity and begins to differentiate in radially elongated cells (Fig. 6g). At the vascular cylinder, the biserial pericycle and the elements of xylem and phloem still in differentiation are observed (Fig. 6e, 6g). Idioblasts with phenolic compounds begin to appear at the inner cortex and at the pith (Fig. 6e).

On the transverse sections 2870 µm from the apex (Fig. 7a), the thick layer of substance secreted by the epidermis becomes thin (Fig. 7a-b). The intercellular spaces of the inner cortex become more conspicuous (Fig. 7c). The endoderm is now completely differentiated, which can be observed by the high level of vacuolation of the cells, as well as the pericycle and vascular elements (Fig. 7c).

At maturity, part of the inner cortex transforms into schizo-lysigenous aerenchyma (Fig. 7d), starting with the dissolution of the middle lamella increasing the intercellular spaces, which then become more evident with the lysis of the cells. The endoderm cells remain with thin walls, and the innermost cell layers of the cortex start to have their walls thickened (Fig. 7e). The root is polyarc, with a great number of metaxylem elements surrounding the pith region (Fig. 7e).

**Discussion**

The underground stem of *Androtrichum trigynum* is a rhizome, as it shows the morphological characteristics described by many authors to define this organ (Font Quer 1982;
Figure 4 – Rhizome transverse sections of *Androtrichum trigynum* (Spr.) Pfeiffer. a. Hypodermis with no starch grains is observed. b. Parenchyma cells of the inner cortex and vascular cylinder with starch grains. c. Note the thickening of the innermost cortical cells and the endodermis and pericycle cells. d. Detail of the innermost cortical cells and the endodermis cells with phenolic compounds on their walls. Cci = innermost cells of the inner cortex; Ci = inner cortex; Ed = endodermis; Ep = epidermis; Fv = vascular bundle; Hp = hypodermis; Id = idioblasts; M = pith; P = pericycle. Bars: 50 μm (c-d), 100 μm (a-b).

Bell 1991; Appezzato-da-Glória 2003). The aerial stem, is spite of being perennial, is classified as reproductive, as it is the axis of the inflorescence or floral scape, as occurs in other Cyperaceae species described by Estelita & Rodrigues (2007). According to these authors, the main anatomical characteristic that distinguishes the stem from the scape in Cyperaceae is the presence of the PTM in the former, as occurs in *A. trigynum*.

The anatomy of the stem and root apexes of *A. trigynum* is similar to what is described for the other Cyperaceae species (Gifford & Bayer 1995; Rodrigues & Estelita 2002, 2004 and 2009). It draws attention, however, to the papillose
epidermal cells of the root of \emph{A. trigynum}, which do not form root hairs, but secrete mucilage, detected with an specific test. For Mauseth (1988), the mucilaginous substance secreted by the apical region of the roots is known as mucigel, which would protect and lubricate the root apex, aiding water and nutrient absorption (Dickison 2000). It is believed that, in \emph{A. trigynum}, mucilage also works as a thermal insulator to the high temperatures that reach the soil, especially in winter, also helping on
Figure 6 – Root apex transverse sections of *Androtrichum trigynum* (Spr.) Pfeiffer. a-d. Sections at 280μm from the root apex. a. General aspect. b. Detail of the substance secreted by epidermal cells (*). c. Outer cortex or hypodermis and inner cortex. d. Inner cortex originated from the meristematic endodermis with schizogenous intercellular spaces (arrows) and vascular elements in differentiation in the vascular cylinder. e-g. Sections at 1610 μm from the root apex. e. General aspect. f. Detail of the epidermal cells and outer cortex. g. Detail of the endodermis and vascular cylinder. Cf – root cap; Ci – inner cortex; Edm – meristematic endodermis; Ed – endodermis; Ep – epidermis; F – phloem; Hp – hypodermis; Id – idioblasts; Mx – metaxylem; P – pericycle; Px – protoxylem. Bars: 100 μm (a, e), 20 μm (b-d, f-g).
Figure 7 – Root transverse sections of *Androtrichum trigynum* (Spr.) Pfeiffer. a. General view. b. Detail of the epidermis and outer cortex. c. Thickening of the walls of the inner cortical cells (arrows). d. Later stage of development, showing schizo-lysigenous aerenchyma, endodermis (arrow) with thin-walled elongated cells and metaxylem elements differentiated. e. Mature root, showing detail of the cortex and vascular cylinder. Cci – innermost cells of the inner cortex; Ci – inner cortex; Ed – endodermis; Ep – epidermis; F – phloem; Hp – hypodermis; Id – idioblasts with phenolic compounds; Mx – metaxylem; P – pericycle; Px – protoxylem. Bars: 100 μm (a, d-e), 50 μm (b), 20 μm (c).
water retention and thus preventing desiccation, as Dickison (2000) suggests.

The intercalary meristem (IM), observed at the stem apex of *A. trigynum*, was also observed in the rhizomes of *Cyperus giganteus* (Estelita & Rodrigues 2007) and *Fuirena umbellata* Rottb. (Rodrigues & Estelita 2009). According to Estelita & Rodrigues (2007), the intercalary meristem (IM) is frequent in leaves and internodes of many monocots, scapes and pedicels. However, it shows higher activity at the scapes, allowing the elongation of the internodes (Fisher 1970). It is believed that the presence of the IM at the apical region of *A. trigynum* rhizome indicates the beginning of floral scape formation, that is, the beginning of the reproductive stage, as seen in other Cyperaceae (Estelita & Rodrigues 2007).

The PTM is a lateral meristem characteristic of monocots (Rudall 1991); it has been described for many plants of this group, and it may occur in herbaceous (Krauss 1948; Sajo 1992) and rhizomatous species (Rudall 1984, 1991; Rodrigues & Estelita 2004, 2009), and in more immature regions of monocots with secondary growth (Fisher & Tomlinson 1972). In Cyperaceae, it was registered for the genera *Scirpus* L. and *Fimbristylis* Vahl. by Rudall (1991), and in the species *Cyperus esculentus* (Gifford & Bayer 1995) and *C. giganteus*, *C. rotundus*, *Fuirena umbellata* Rottb., *Hypolitrum rotundus*, *Fiu rena umbellata* Rottb., *Hydrocharis morus-ranae* L. (Hydrocharitaceae), Seago *et al.* (2000) in *Pontederia cordata* L. (Pontederiaceae), Melo-de-Pinna & Menezes (2003) in *Richterago Kuntze* (Asteraceae), Rodrigues & Estelita (2004) in *Cyperus giganteus*.

According to Melo-de-Pinna & Menezes (2003), at the roots of *Richterago Kuntze*, the meristematic endoderm experiences successive anticlinal and periclinal divisions to form the inner cortex, remaining as a meristematic layer until its complete differentiation, when the Casparian strip arises. This is a striking feature for recognition of the endoderm (Van Fleet 1961). In stems, many authors called it “endodermous layer” as they could not observe the Casparian strip (Tomlinson 1969; Gifford & Bayer 1995). In the studied species, Casparian strips were not observed in the endoderm cells, neither on the roots nor on the rhizome; however, it can be identified by its morphological features. At the roots, initially in meristematic regions, the endoderm can be recognized by having meristematic activity. At posterior stages, the endoderm consists of radially elongated cells, morphologically distinguished from the other layers, such as in *Actinocephalus* species studied by Scatena *et al.* (2005). On *A. trigynum* rhizome, the endoderm can be recognized by having cell features that distinguish from the other cortical cells, such as cell shape and parietal thickening, such as observed in other Cyperaceae species (Eiten 1969; Estelita 1993; Chabbi *et al.* 2000; Arruda & Neves 2005 and Prata *et al.* 2007).

In monocots, the endoderm may present four development stages, starting with the Casparian strip, followed by the deposition of suberin lamella, thickening of lignin and, lastly, deposition of phenolic compounds, as observed by Rodrigues & Estelita (2004) in the roots of *Cyperus giganteus*. In the roots of *A. trigynum*, the endoderm cells remain with their thin walls,
and the thickening occurs in the walls of the innermost cortical cells, which was also observed by Rodrigues & Estelita (2004) in *C. giganteus* roots. In the rhizome of *A. trigynum*, the walls of the endoderm cells lignify at maturity, as well as the walls of the inner cortical cells, which also present deposition of phenolic compounds.

The hypodermis, observed on the roots and rhizomes of *A. trigynum*, is common in Cyperaceae species, and originates independently, from the fundamental meristem (Rodrigues & Estelita 2002, 2004), as also observed by Seago & Marsh (1989) in *T. glauca*. Attention is drawn to some species of Cyperaceae, in which the hypodermis may occur even at the leaves (Metcalfe 1971), such as in *Cyperus corymbosus* Rottb. and *Remirea maritima* Aubl. (Estelita 1993). The number of layers, thickening and composition of the hypodermal cells may vary (Seago & Marsh 1989). The Casparian strip may occur on the outermost layer, which is called exodermis by authors such as Van Fleet (1950). In this species, no modifications on the walls of hypodermal cells were observed, as well as in *H. morsus-ranae*, observed by Seago et al. (1999). At the roots of *A. trigynum*, however, the outermost layer of the hypodermis showed different content in the early stages of differentiation, similar to the content of epidermal cells, indicating a possible participation of this layer in the process of mucilage synthesis. In roots of *T. glauca* (Seago & Marsh 1989) and *H. morsus-ranae* (Seago et al. 1999a), the hypodermis, as well as the thickened inner cortical cells, sustains the aerenchyma, a function that can also be attributed to the hypodermis in the roots of *A. trigynum*, which justifies the use of the term.

The inner cortex of *A. trigynum* roots differentiates in lysigenous aerenchyma, as defined by Seago *et al.* (2005). However, these same authors classify the tangential lysigenous type for Cyperaceae, where the separation and collapse of the cells occur tangentially among the intact lines of cells. On the other hand, in *A. trigynum*, we can observe the formation of lysigenous-radial aerenchyma, where the separation and collapse of cells occur radially among the intact radial lines of cells. The aeriferous system of *A. trigynum* roots was also observed in other species from saline environments, such as *Jaumea carnosa* (Less.) Gray (Omer & Moseley 1981) and *R. maritima* (Estelita 1993). According to Cordazzo *et al.* (2006), aerenchyma is crucial in this environment, as it allows for gas exchange and maintains the aerobic environment, having the species occupy bigger areas during the rainy season, when competition with other perennial species with less capability to resist the floods diminishes.

The rhizome of *A. trigynum* shows a great quantity of starch grains. This characteristic is common in thickened rhizomes, and it is considered an adaptive strategy of plants from xeric environments (Braendle & Crawford 1982).

The results obtained in this work show that the underground system of *A. trigynum* presents anatomical features that can be considered common to Cyperaceae species, such as the presence of a primary thickening meristem at the rhizome, meristematic endoderm, aerenchyma originated from the roots and presence of hypoderm in both organs. Other characteristics such as thickening and lignification of cells, high concentrations of starch grains and phenolic substances, large intercellular spaces and mucilage at the roots, can be regarded as potential strategies to adapt to dunes environment.

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