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Development and function of root hairs in *Acianthera* Scheidw. (Orchidaceae: Pleurothallidinae)

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ABSTRACT

Background: root hairs are long tubular-shaped outgrowths from root epidermal cells. They have been studied as to several vascular plant species, however, in Orchidaceae, root hairs are scarcely reported in the literature and their function has not yet been clarified. Objective: the present study was aimed at describing the development of such structures in Orchidaceae. We also recorded the occurrence of root hairs in *Acianthera*, so as to elucidate the role played by them. Materials and Methods: roots of 13 *Acianthera* taxa were assessed, comprising seven out of the 10 sections of the genus. Histological slides of the roots were stained with alcian blue and basic fuchsin or toluidine blue and then they were analyzed under light microscopy and fluorescence microscopy. Furthermore, the samples were subjected to histochemical tests such as PAS, ruthenium red and acidified phloroglucinol. The root samples were also processed and analyzed under Scanning Electron Microscopy. Results: the *Acianthera* species studied herewith exhibited bi-stratified velamen. We observed that either simple- or spiral-shaped root hairs develop in the epivelamen, but only in the roots facing the substrate. Moreover, the root hairs of *Acianthera* are not ephemeral, but prevail even after the complete root development. Both simple- and spiral-shaped trichomes showed a positive reaction to acidified phloroglucinol, PAS and ruthenium red. Conclusion: based upon our findings, we may conclude that the root hairs in Orchidaceae arise from the outermost layer of the velamen, that is, on the root surface in direct contact with the substrate. We may infer that the spiral-shaped root hairs show secondary cell walls and they are the final differentiation stage in Pleurothallidinae. The simple and spiral root hairs play a key role as to the attachment of orchids to the substrate as well as the water and nutrient uptake.

INTRODUCTION

Root trichomes, also known as root hairs are long tubular-shaped outgrowths from root epidermal cells. These structures provide better plant-soil fixation and increase nutrient uptake (Gilroy & Jones, 2000). Root hairs are found close to the apical zone (Dickison, 2000) and their development occurs in four stages, namely: cell-fate specification, initiation, apical growth and maturation (Gilroy & Jones, 2000; Forde & Lorenzo, 2001). Research studies about root hair development in angiosperms have been primarily focused on both physiological (Peterson & Farquhar, 1996; Forde & Lorenzo, 2001; Ma *et al*., 2001) and molecular aspects (Gilroy & Jones, 2000; Shi & Zhu, 2002; Mozahim *et al*., 2014).

Orchidaceae roots are overlaid by multiple epidermal layers (velamen), whose primary functions are related to the absorption and storage of water and nutrients as well as root protection (Benzing *et al*., 1983). Moreover,

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a root velamen is a typical characteristic of epiphytic species which belong to the Orchidaceae family, although it also occurs in terrestrial orchids (Toscano de Brito & Cribb, 2005; Stern, 2014).

The presence of root hairs in Orchidaceae is rarely mentioned in the literature (Leitgeb, 1865; Groom, 1893; Morris *et al*., 1996; Carlsward *et al*., 2006; Chomicki *et al*.; 2014; Stern, 2014), let alone their morphological types. Spiral- and simple-shaped root hairs have been reported for some species of this family, however, root hair distribution patterns in the root epidermis are little known and the lack of ontogenetic and physiological studies difficults the understanding about the role of root hairs (Bernal *et al*., 2015).

Acianthera Scheidw., is an important genus relating to epiphyte species. It belongs to the subtribe Pleurothallidinae (Orchidaceae) and comprises 291 species distributed throughout South America (Karremans, 2016). Anatomical studies as to the roots of *Acianthera* are restricted to a few species (Pridgeon, 1982; Scatena & Nunes, 1996) and root hairs have not been reported whatsoever. In Pleurothallidinae, root trichomes were reported solely to *Dresslerella* (Pridgeon & Williams, 1979).

Given the scarcity of reports on the development of root hairs in Orchidaceae, the present study was aimed at investigating and describing the development of such structures. We also recorded the occurrence of root hairs in *Acianthera*, so as to elucidate the role played by them.

MATERIAL AND METHODS

Roots of 13 *Acianthera* taxa were analyzed, comprising seven out of the 10 sections of the genus (Table 1). We collected the roots that were growing attached to the substrate and also the roots that were not in direct contact with it. For the light microscopy analysis, transverse and longitudinal sections of fresh material or fixed in FAA 50% (Johansen, 1940) were obtained by hand, with the aid of razor blades and stained with alcian blue and basic fuchsin (Luque *et al*., 1996). Samples were also embedded in historesin (Leica Historesin Kit), according to the manufacturer's instructions, and then stained with toluidine blue (O'Brien *et al*., 1964). The samples were then subjected to the following histochemical tests: (i) ruthenium red for acidic mucilage (Gregory & Baas, 1989); (ii) PAS to stain total insoluble polysaccharides (McManus, 1948); (iii) acidified phloroglucinol to stain lignin (Foster, 1949). Furthermore, images were captured using Olympus SC30 microscope coupled with digital camera and analySIS-getIT software.

The autofluorescence of root hair cell walls was tested by fluorescence microscopy analysis (Liu *et al*., 1994), using Texas Red (RFP) filter with an Olympus BX51 microscope coupled to a device camera (Olympus, DP72). Furthermore, samples were also visualized under confocal microscope Nikon A1RSiMP (Nikon Corp., Tokyo, Japan) and further imaging analysis was done by using Nikon software (NIS-Elements 4.20).

All samples were fixed in FAA 50%, then they were dried to critical point, gold coated and viewed under Scanning Electron Microscopy (SEM). Analysis and SEM image recording were carried out by using Tescan Vega3 LMU.

Table 1: List of *Acianthera* Scheidw. taxa studied (Orchidaceae: Pleurothallidinae).

Taxon	Section	Voucher information
Acianthera aphtosa (Lindl.) Pridgeon & M. W. Chase	*Sicariae*	Koene, F.M. 009 UPCB
Acianthera atropurpurea (Barb. Rodr.) Chiron & van den Berg	*Pleurobotryae*	Almeida, A.B.R. 020 HUCP
Acianthera crepiniana (Cogn.) Chiron & van den Berg	*Pleurobotryae*	Almeida, A.B.R. 05 HUCP
Acianthera fenestrata (Barb. Rodr.) Pridgeon & M. W. Chase	*Cryptophoranthae*	Almeida, A.B.R. 036 HUCP
Acianthera gracilisepala (Brade) Luer	*Acianthera*	Almeida, A.B.R. 01 HUCP
Acianthera hatschbachii (Barb. Rodr.) Chiron & van den Berg	*Pleurobotryae*	Almeida, A.B.R. 03 HUCP
Acianthera hatschbachii (Barb. Rodr.) Chiron & van den Berg	*Pleurobotryae*	Kersten, R.A. HUCP18411
Acianthera luteola (Lindl.) Pridgeon & M. W. Chase	*Sulcatae*	Almeida, A.B.R. 011 HUCP
Acianthera mantiquyrana (Barb. Rodr.) V. T. Rodrigues & F. Barros	*Pleurobotryae*	Almeida, A.B.R. 02 HUCP
Acianthera octophrys (Rchb. f.) Pridgeon & M. W. Chase	*Tomentosae*	Toscano de Brito, A.L.V. 3410 UPCB
Acianthera prolifera (Herb. ex Lindl.) Pridgeon & M. W. Chase	*Sicariae*	Almeida, A.B.R. 014 HUCP
Acianthera pubescens (Lindl.) Pridgeon & M. W. Chase	*Acianthera*	Almeida. A.B.R. 04 HUCP
Acianthera saurocephala (Lodd.) Pridgeon & M. W. Chase	*Acianthera*	Almeida, A.B.R. 027 HUCP
Acianthera saurocephala (Lodd.) Pridgeon & M. W. Chase	*Acianthera*	Almeida, A.B.R. 07 HUCP
Acianthera teres (Lindl.) Borba	*Tricarinatae*	Almeida, A.B.R. 015 HUCP

Results:

The analyzed species showed bi-stratified velamen with endovelamen formed by thin-walled cells and epivelamen formed by cells whose periclinal inner-wall had strongly lignified thickening (Fig. 1A). Furthermore, the root hairs were observed in the outermost layer of the velamen (Fig. 1B).

Trichomes were observed throughout the whole length of the root, close to the apex and distal zone of the root (Fig. 1C). Root hairs develop facing the substrate as seen in Figures 1C, 1F and 1J, in which the remaining substrate can be observed attached to the root tips. Regions of the roots without direct contact to the substrate showed no root hairs (Fig. 1B).

In regions near the apex, we observed protuberant epidermal cells on the periclinal outer wall (Fig. 1D) as well as short root hairs free from the substrate. As to distal regions, we observed root hairs in apical growth with

the cytoplasm concentrated at the apex (Fig. 1E, arrow). The thoroughly developed root hairs were attached to the substrate (Fig. 1C, 1F) and they showed a whole cell wall or disrupted in spiral shape (Fig. 1G, 1H). There has been a histochemical reaction of the root trichomes to acidified phloroglucinol and autofluorescent cell walls when analyzed under fluorescence microscopy (Fig. 1I). Root hairs reacted positively to PAS and ruthenium red (Fig. 1J), indicating the presence of total insoluble polysaccharides and acidic mucilages.

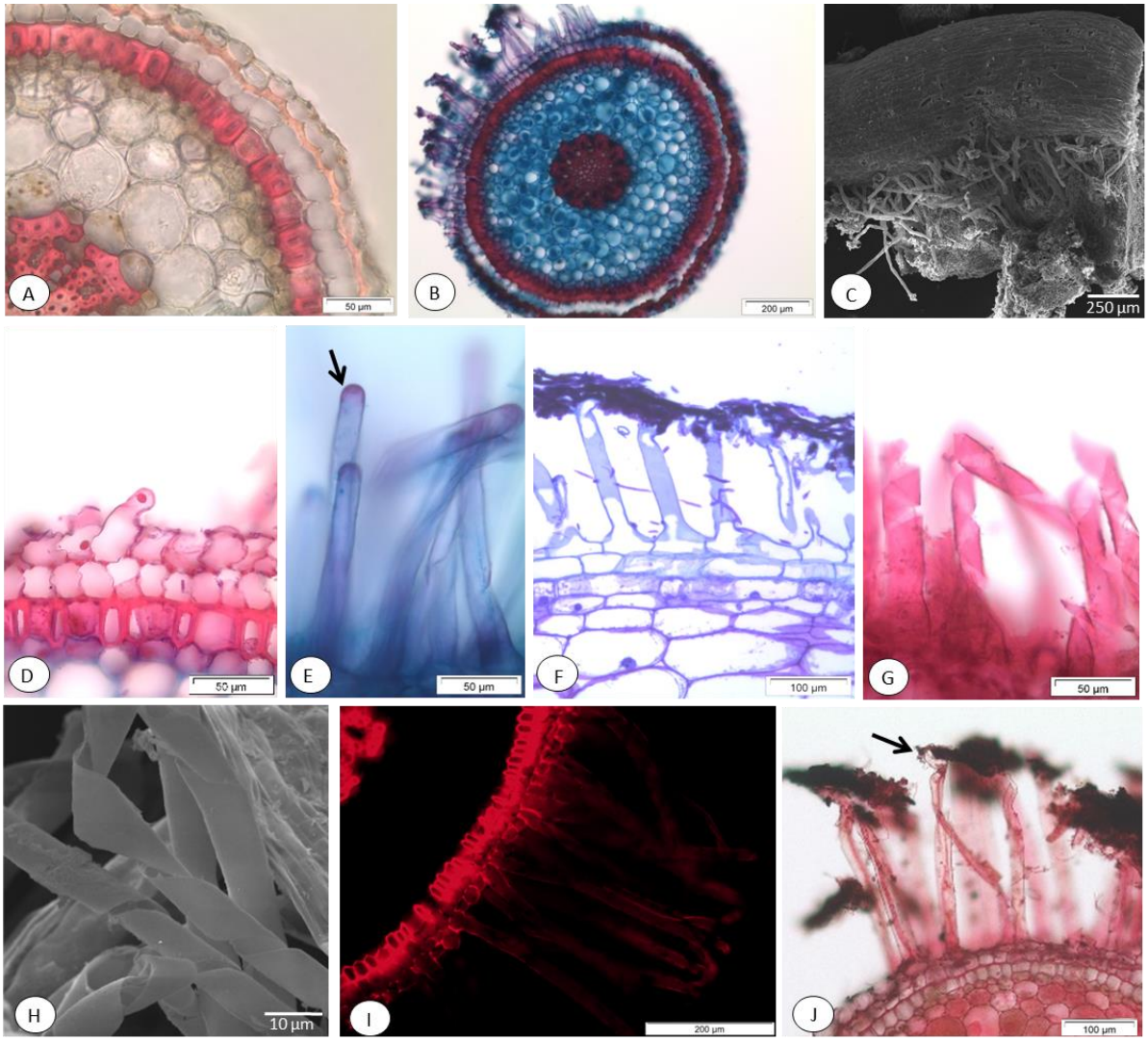

Fig. 1: *Acianthera* roots under light microscopy (A-B, D-G, J) and SEM (Scanning Electron Microscopy) (C, H). A – Bi-stratified velamen of *Acianthera luteola* showing lignified thickening in the periclinal inner-wall evidenced by the acidified phloroglucinol test. B – Grown root hairs of *A. crepiniana*. C – Root overview of *A. aphtosa* with root hairs solely facing the substrate. D – *A. gracilisepala* root hairs in the early stage of development. E – *A. crepiniana* root hairs in apical growth stage with cytoplasm concentrated at the apex (arrow). F – *A. aphtosa* longitudinal root section exhibiting simple-shaped trichomes grown and attached to the substrate. G – *A. crepiniana* spiral-shaped root hairs evidenced by PAS test. H – *A. pubescens* spiral trichomes. I – *A. aphtosa* root hairs under fluorescence microscopy. J – *A. aphtosa* root hairs attached to the substrate (arrow), evidenced by the ruthenium red test

Discussion:

All analyzed roots exhibited trichomes in the apical region as well as within the regions in direct contact with the substrate. Root hairs develop in the regions near the root apex and are mostly found in vascular plants, although ephemeral, but restricted to the apical region (Dickison, 2000; Evert, 2006). However, considering the location of the root hairs observed in *Acianthera*, these structures are persistent throughout root development, since they are found even in remote regions of the root apex.

Root hairs arise from epidermal cells, also known as trichoblasts. During the ontogenesis of these structures (first stage), it is determined whether or not the epidermal cell will become a trichoblast (Forde & Lorenzo,

2001). The second stage of root hair development is called initiation, which is characterized by protrusion formation within one of the cell wall sides (Gilroy & Jones, 2000) as shown in Figure 1D. It indicates that the root hairs in Pleurothallidinae arise from the outermost layer of the velamen.

The growth stage of root hairs, also called apical growth, is a genetic process and physiologically different from the initiation stage (Gilroy & Jones, 2000). In terrestrial plants, the root development as well as the number of root hairs and their length may vary according to the occurrence of inorganic nutrients in the soil (Forde & Lorenzo, 2001; Ma *et al*., 2001, Nozulaidi *et al*., 2015; Idress *et al*., 2016). However, in epiphytic species of Orchidaceae, the development of root hairs seems to be related to the aerial roots in contact with the substrate since their development only occurs on the root surface facing the substrate, which favors the attachment of the roots and the orchid itself (Groom, 1893; Chomicki *et al*., 2014; Stern, 2014).

Root hairs of epiphytic orchids can be simple (Pridgeon & Williams, 1979), branched or spiral-shaped (Leitgeb, 1865). We observed simple- and spiral-shaped root hairs in *Acianthera*, but they differ from the spiral-shaped trichomes described by Leitgeb (1865) as to other species of Orchidaceae, although they resemble the spiral-shaped root hairs of *Eria* sp. (Janczewski, 1885) and Spirantinae (Bernal *et al*., 2015). Based on our findings about root hairs in different stages of development, we may infer that the spiral-shaped root hairs are the final differentiation stage in Pleurothallidinae since the outgrowth of these structures provides the disruption of the spiral cell wall (Lersten & Curtis, 1977).

Unlike the spiral trichomes found on leaves of Rosaceae, Betulaceae and Rubiaceae, which maintain continuous inner wall and possibly retain the protoplast at maturity (Lersten & Curtis, 1977), the spiral root trichomes recorded for *Acianthera* as well as root hairs of Spirantinae (Bernal *et al*., 2015) showed completely disrupted spiral walls and do not retain the protoplast at the final development stage. Thus, we believe that the spiral cell walls of these root hairs can provide better attachment and plasticity to the roots of epiphytic orchids, preventing their detachment from the substrate by mechanical stress.

Lersten and Curtis (1977) suggested that the disrupted outer wall is the original primary wall of spiral-shaped root hairs. Nevertheless, the positive reaction of the root trichomes to acidified phloroglucinol and autofluorescence presented by the cell walls of some simple- and spiral-shaped trichomes indicates the occurrence of lignin deposition in the cell walls of simple-shaped trichomes and consequent disruption of the cell wall. Therefore, one may infer that the spiral root trichomes found in Pleurothallidinae have secondary spirally disrupted cell walls.

As a matter of fact, the presence of PAS-positive substances overlying the roots of the analyzed species indicates that they act as adhesive roots, by enabling them to attach to the substrate (Badalamenti *et al*., 2015), and the mucilage is found to be forming a sort of 'membrane' in the roots and root hairs, aiding the water and nutrient uptake (Oades, 1978). These results corroborate that the root trichomes found in Pleurothallidinae play a key role as to the attachment of orchids to the substrate, as well as, the absorption of water and nutrients by the root.

Conclusion:

Our findings corroborate that root hairs play a key role in the uptake of water and nutrients as well as plant attachment to the substrate. These structures are not ephemeral, but can rather prevail even after the complete root development. The very location of root hairs in early development indicate that they originate from the outermost layer of the velamen and their development is related to aerial roots in contact with the substrate. Spiral-shaped trichomes are the final differentiation stage of root hairs and they arise from epidermal cell outgrowths, deposition of lignified secondary wall as well as the disruption of spiral cell wall.

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