Flora 230 (2017) 66–74

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

The velamen of epiphytic orchids: Variation in structure and correlations with nutrient absorption

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ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form 7 March 2017
Accepted 13 March 2017
Edited by Hermann Heilmeier
Available online 16 March 2017

Keywords:
Nutrient uptake
Orchidaceae
Passage cells
Pectins
Root anatomy
Velamen structure

ABSTRACT

Roots of epiphytic orchids typically possess a velamen radicum, a multiple epidermis composed of dead cells. It is largely unexplored how the structure of the velamen, the cortex and vascular cylinder facilitate the flow of water and nutrients towards the plant. Up to now, structural root features were rarely correlated with functional attributes. In this study, we compare anatomical features with nutrient uptake rates (specifically of phosphorus and rubidium) of roots of 18 taxa of epiphytic orchids. The relative proportion of the velamen of the cross-sectional area varied from 11 to 97%. Species with the largest relative velamen area facilitate the flow of water and nutrients, and species with larger relative proportion of vascular cylinder and cortex have a greater number of protoxylem strands and passage cells of the endodermis. The number of passage cells in the endodermis and of differential wall thickenings in cortical cells may play an important role in apoplastic and symplastic flow. The velamen pectic matrix, with negative charge, can retain cations (like Rb^+), while a higher number of strands in the xylem, aligned with passage cells of the endodermis, could facilitate the entry of these ions into the vascular cylinder.

1. Introduction

The epiphytic environment imposes a number of restrictions to plants. Many of the anatomical features commonly found among epiphytes are related to the uptake of water and nutrients, such as absorbing scales in the Bromeliaceae or the velamen radicum in Orchidaceae (Withner, 1959; Benzing, 1990; Zotz and Hietz, 2001; Laube and Zotz, 2003). Although the availability of water and nutrients is sporadic and depends on atmospheric sources (Benzing, 1990), growth in tree crowns ensures an environment with an improved supply of light (Tsavkelova et al., 2001). It is known that approximately 10% of all vascular plants are epiphytes, the Orchidaceae being particularly noteworthy because around 70% of all species are epiphytic (Zotz, 2013). This success in tree crowns is arguably due to anatomical and physiological strategies that compensate for the scarcity of water and nutrients, promoting in the effective absorption and use of these resources when intermittently available (Scatena and Nunes, 1996).

Among the adaptive features of orchid roots, the presence of velamen stands out (Zotz and Hietz, 2001). The velamen is located externally to the exodermis, the outer layer of the cortex, and constitutes a multiple epidermis composed of dead cells (Pridgeon, 1987). Initially, Went (1940) proposed an important role of the velamen in capturing and immobilizing solutions that arrive at the root via stem flow, and recent experimental evidence provides support for this notion (Zotz and Winkler, 2013). In addition to the function of water absorption, the velamen also reduces the loss of water at times of low availability of water and confers mechanical protection for the root (Pridgeon, 1987; Benzing, 1996).

Internally to the velamen, the cortex consists of parenchymatic cells. In Orchidaceae there are different types of parietal thickenings, i.e. reticulated, uniform or phi forms (Stern, 1999; Stern and Judd, 2001; Moreira et al., 2013). The exodermis and endodermis (outermost and innermost layers of the cortex, respectively) delineate the cortical parenchyma in these roots, and the presence of an endodermis acts as boundary between the cortex and the vascular cylinder. Exodermis and endodermis are formed by cells that can have Casparian strips, be highly lignified and sometimes dead at maturity, alternating with passage cells which remain alive

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http://dx.doi.org/10.1016/j.flora.2017.03.009
0367-2530/

(Pridgeon, 1987; Trépanier et al., 2008). The lignified cells (or with Casparian strips) in these layers act as a barrier for the movement of ions and other substances through the apoplast (Esnault et al., 1994; Ma and Peterson, 2003), while the passage cells are the main cells capable of symplastic movement (Peterson and Enstone, 1996). These cells allow water and nutrient absorption (Peterson and Enstone, 1996), and are responsible for the selectivity of solutes to be transported to the vascular cylinder (Peterson, 1988; Trépanier et al., 2008).

This study wants to relate the investment of Orchidaceae roots in velamen and/or in other internal structures to the functions assigned to the passage cells and velamen found in the cited literature, specifically the absorption of nutrients. We focus on the relationship of the thickness of the velamen and the number of passage cells (of both the endodermis and exodermis) as structural characteristics on the one hand and the rate of absorption of phosphorus and rubidium on the other hand for a set of 18 taxa of epiphytic orchids.

2. Material and methods

Individuals of *Bifrenaria harrisoniae* (Hook.) Rchb.f., *Catasetum planiceps* Lindl., *Cattleya skinneri* Bateman *alba*, *Caularthron bilamellatum* (Rchb.f.) R.E. Schult, *Dendrobium fimbriatum* Hooker, *Dendrobium nobile* Lindl., *Dendrobium nobile* hybrid, *Dimerandra emarginata* (G. Mey.) Hoehne, *Doritis pulcherrima* Lindl., *Encyclia ghillanyi* Pabst, *Epidendrum ciliare* L., *Epidendrum nocturnum* Jacq., *Gongora unicolor* Schltr., *Miltonia bluntii* Rchb.f., *Phalaenopsis cornu-cervie* Blume & Rchb.f., *Phalaenopsis* hybrid, *Oncidium* sp. and *Trichoglottis bipunctata* (C.S.PP. Parish & Rchb.f.) Tang & F.T. Wang were obtained from the Botanical Garden of the University of Marburg (Germany) or from collections in Panama, and acclimated in a greenhouse of the University of Oldenburg (Germany), at a temperature of 26/20 °C (day/night) and a relative humidity of ca. 65%. Fragments of aerial roots were submitted to tests of nutrients absorption (phosphorus and rubidium) in the Radionuclide Laboratory of the University of Oldenburg and parallel samples were fixed in 4% paraformaldehyde (Roland and Vian, 1991). The anatomical analyses were performed in the Laboratory of Plant Anatomy and Development of the Universidade Federal de Uberlândia, Brazil.

2.1. Experiments of nutrient absorption

Nutrient absorption was assayed similar to the procedure described in Zotz and Winkler (2013) using the following radioisotopes: carrier free ^{32}P phosphoric acid (Hartmann Analytic, Germany), containing 37 MBq in 100 μl water (initial specific activity: 5.5 MBq nmol^{-1} ^{32}P); aquatic solutions of $^{86}RbCl$ (Hartmann Analytic, Germany) with a specific activity of 720 MBq mg^{-1} Rb^+ and aquatic solutions of [^{14}C(U)-D-glucose] with a specific activity of 13.3 GBq mmol^{-1}. The radioisotope of rubidium is a well-established analytical analogue for potassium, because the uptake kinetics of K^+ and Rb^+ are generally comparable (Läuchli and Epstein, 1970). Aliquots of labelled substances with an initial activity of 0.15 mBq in 5 ml incubation solution were used in uptake experiments with the 18 different taxa. The uptake of phosphate was measured in solutions containing radioactive labelled ^{32}P phosphate and unlabelled phosphate to give a final substrate concentration of 15 μM. Radioactive, labelled ^{86}Rb and unlabelled RbCl were mixed to obtain a similar final concentration of 15 μM. Such concentrations are within the range of concentrations naturally occurring in rainwater (e.g. Benzing, 2000).

Four root segments of each taxon, excised at 4 cm from the root tip, were submerged in solutions of incubation in test tubes, and absorption rates were determined by the decrease of the radioactivity in aliquots of 10 μl of the solutions of incubation (with pH 6.1) after standardizing the volume of liquid, adding the solution not labelled to the initial volume of incubation, mixing with Pasteur pipettes. The velamen was removed just above the submerged portion to prevent the passive diffusion in this tissue.

The cumulative absorption of phosphate and rubidium was calculated from the decrease of the radioactivity in the solution of incubation with a constant volume, according to the following equation:

$$Absorption(\text{nmol}) = P_\Sigma - (dpm_{t0}/dpm_{t1} \times P_\Sigma),$$

where P_Σ is the initial quantity of substrate in the solution (nmol); dpm_{t0} is the counting rate in 10 μl at beginning of absorption and DPM_{t1} the counting rate in 10 μl at the end of the period of absorption. The rates were expressed against root length (cm).

2.2. Structural analysis

For anatomical comparisons of the roots, histological slides of transverse hand cuts were prepared 3 cm from the apex. The material was clarified in 50% sodium hypochlorite (Kraus and Arduin, 1997) and stained with 1% alcoholic solution of safranin and 0.5% Astra blue (1:9 v/v) (Bukatsch, 1972). The slides were assembled with gelatin glycerinated of Kaiser (Johansen, 1940) and the material photographed under light microscope Leica® DM500 equipped with digital camera Leica® ICC50 HD.

For each species, four individuals were analyzed when available (see analysis of results). For each individual, 15 transverse cuts were performed. The diameter, the number of layers, the thickness and the cross-sectional area occupied by the velamen, and the cross-sectional areas of vascular cylinder and the cortex were determined at three different points of a given cut. The cross-sectional areas of different tissue types were expressed relative to the total area. The total number of cells of the exodermis and endodermis, the thickness of the cell walls of the exodermic cells and the number of passage cells were quantified as were the ratios of passage cells to the other cell components of the endodermis and exodermis. In addition, we counted the number of strands of xylem and phloem. For all counts and measurements, IMAGE J 1.x software (National Institute of Health, USA) was used.

2.3. Histochemical analysis

The presence of proteins was used to identify living cells and tissues. Transverse sections were incubated in 1% bromophenol blue solution (w/v) for 10–15 min (Durrum, 1950), washed in 50% ethanol (v/v) and assembled in distilled and deionized water. Pectins were detected incubating transverse sections in 50% ethanol, and staining with 0.2% ruthenium red (w/v) for 10 min (Chamberlain, 1932; modified).

2.4. Data analysis

Structural data were analyzed with the averages of the individuals in each species. For *Caularthron bilamellatum, Cattleya skinneri alba, Dendrobium fimbriatum, Epidendrum ciliare* and *Miltonia bluntii*, there was only material available from a single individual. For *Phalaenopsis* hybrid, *Gongora unicolor* and *Trichoglottis bipunctata* there were only two available individuals of each taxon. In all other cases, four individuals were used.

We evaluated the correlation between variables using Pearson test for parametric data. The same test was used to compare the structural characteristics with the rates of absorption of rubidium and phosphorus. All tests were performed using SYSTAT 10.2 soft-