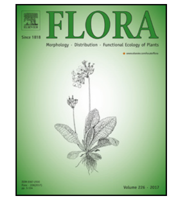
Flora 226 (2017) 17–28

Contents lists available at ScienceDirect

Flora

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



Comparative anatomy of aerial and substrate roots of *Acampe praemorsa* (Rox.) Blatt. & McCann

Thangavelu Muthukumar*, Ayyasamy Kowsalya

Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore 641046, Tamilnadu, India

ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form 4 November 2016
Accepted 4 November 2016
Edited by Alessio Papini
Available online 7 November 2016

Keywords:
Branched root hairs
Exodermal proliferation
Mycorrhiza
Pelotons
Water cells
Velamen

ABSTRACT

Roots of epiphytic orchids grow in air, attached to bark or in soil. Although roots growing under varied environmental conditions exhibit significant differences in morphology and anatomy, such information is lacking for orchids. Therefore we investigated the anatomical differences in roots of *Acampae praemorsa* (Rox.) Blatt. & McCann growing positively or negatively geotropic in air, soil or attached to the bark. Roots were sectioned and stained with various dyes and the cellular details were examined and measured. In addition, mycorrhizal colonization in roots was also examined. Roots of *A. praemorsa* had *Vanda*-type velamen. Root hairs developed only on roots that were growing in soil or on regions of the roots that were attached to the bark and were branched in terrestrial roots. Cells constituting different root tissues significantly varied in their size and wall thickening. Aerial roots exhibited characters adaptive to drought like thick velamen, increased cortical cell layers, and endodermis with much-thickened walls. Water cells were present in all types of roots. In contrast, aeration units occurred adjacent to the endodermis in roots attached to bark. The number of passage cells per xylem arch in terricolous roots was more than other root types. The vascular tissues were embedded in sclerenchymatous tissue. The pith was sclerenchymatous in substrate roots, whereas it was parenchymatous at the central region in aerial roots. The variation in the anatomy of roots growing in different microhabitats clearly indicates the plasticity of epiphytic orchid roots to capture nutrients and respond to abiotic stress.

1. Introduction

Roots, the primary organ for plant fixation and absorption of water and nutrients for plant growth and metabolism has received little attention than shoots. Roots of certain plant taxa are unique as they enable plant's survival in non-terrestrial habitats. This is particularly true for taxa belonging to Orchidaceae, one of the largest families of flowering plants. Though, this plant family consists of more than 25,000 species in 780 genera, the majority of the taxa (~80%) are epiphytes (Dressler, 1993; Pridgeon et al., 2009). Epiphytic orchids have aerial roots that absorb moisture from the atmosphere. This unique ability of aerial roots is due to the presence of one to several layers of the specialized epidermis called velamen. In addition, the roots also attach the plant onto the substrate and absorb water and minerals from it. Some aerial roots of epiphytic orchids exhibit negative geotropism that was presumed to perform the function analogous to pneumatophores. The negatively geotropic aerial roots also function as 'litter traps'. The litter accumulated in these traps decomposes providing minerals for plant growth. The aerial roots and roots that are attached to substrates in orchids can grow and reach the soil (Porras-Alfaro and Bayman, 2007).

It is generally believed that the anatomy of the plant is usually less affected by environmental conditions than morphological characters (Ponert et al., 2016). Therefore, they may provide valuable clues for taxonomic identifications and assessing phylogenetic relationships between taxa. Nevertheless, it is well known that environmental factors can influence root structure to a great extent. For example, the quantity of aerenchyma in roots generally increases with an increase in soil moisture. In plants, development of any negatively geotropic structure that is not surrounded by a medium induces a tension and anatomical modifications in these structures to counteract the tension (e.g., tension wood in branches). However, unlike shoots, terrestrial roots are less stressed as they are surrounded by a medium. It is, therefore, interesting to see the anatomical modifications of aerial roots that grow negatively geotropic. Similarly, structural changes may occur when aerial roots enter the soil where it encounters an environment that is less variable than the atmosphere. Although, the root anatomy of several epiphytic taxa have been examined (Kaushik, 1983; Singh, 1986; Oliveira and Sajo, 1999; Carlsward et al., 2006; Moreira and Isaias, 2008; Pedroso-de-Moraes et al.,

* Corresponding author.
E-mail addresses: tmkum@yahoo.com, tmk5_99@yahoo.com (M. Thangavelu).

http://dx.doi.org/10.1016/j.flora.2016.11.001
0367-2530/

2012; Vattakandy et al., 2013; Silva et al., 2015) the anatomical variations in the different types of roots in epiphytic orchids is not known. Pedroso-de-Moraes et al. (2012) studied the root anatomy of *Catasetum discolor* (Lindl.) Lindl., growing on terrestrial and epiphytic habitats, but failed to distinguish habitat induced anatomical variation in the roots. Stern and Judd (1999) described the anatomy of aerial and terrestrial roots of 11 *Vanilla* vines. Notable differences were observed in the endodermis and pericycle wall thickenings, tissues in which vascular tissues were embedded, nature of the pith cells, primary xylem points and metaxylem diameter between the aerial and terrestrial roots (Stern and Judd, 1999).

The monopodial epiphytic genus *Acampe* consists of around eight species distributed mostly in Asia with one species occurring in tropical Africa (La Croix et al., 1991; The Plant List, 2013). Among these, *Acampe praemorsa* (Rox.) Blatt. & McCann occurs in India, Sri Lanka, and Seychelles. In India, this species is commonly found in the Western Ghats (Abraham and Vatsala, 1981). Senthilkumar et al. (2000) examined the mycorrhizal association in *A. pramerosa* and showed that the colonization was restricted to the outer and inner layers of the cortex. In addition, they also reported the occurrence of yeast cells on the digesting pelotons in the cortical cells.

Radicular anatomy of a few species of *Acampe* has been studied. In their comparative anatomical and stomatal study of orchids belonging to Vandoideae, Vattakandy et al. (2013) examined roots of *Acampe rigida* Roxb. The roots to consisted of an uniseriate ectovelamen and 6–8 layered endovelamen, radially elongated exodermis with ∪-shaped walls, cortex 10–13 layered; presence of raphides, endodermis with O-shaped thickening, numerous vascular bundles buried in the sclerenchymatous tissue, pith made up circular cells with no intercellular spaces (Vattakandy et al., 2013). In their comparative studies on the vegetative anatomy and systematics of angracecoids of Vandeae, Orchidaceae Carlsward et al. (2006) also examined roots of *Acampepapillosa* (Lindl.) Lindl. The roots contained uniseriate ectovelamen of isodiametric cells and a radially arranged endovelamen without any distinct thickenings. Exodermal cells were O-thickened with or without proliferations. Cortex was 17 cells wide containing water storage cells. The endodermis was O-thickened, vascular cylinder 12–14 arched, and pith parenchymatous. Algal cells present. As *Acampe* produces roots that occupy different microhabitats it would be interesting to see environment-induced changes in the root system of the plant. This study was carried out with the objective of examining the microhabitat induced variation in the root anatomy of an *Acampe* species. The observation of the present study could also provide insight to the plastic characteristic of the root system and modifications for maximum resource capture in orchids.

2. Materials and methods

Different types of *A. praemorsa* roots for this study were obtained from a home garden from Coimbatore (11°01′N and 96°93′E, altitude 410 m a.s.l), Tamilnadu, India during January 2016. The city of Coimbatore has a tropical wet and dry climate according to the Köppean climate classification. Though the city receives its 700 mm rainfall from both southwest monsoon (June–August) and northeast monsoon (October–December), the later is more vigorous. The mean maximum temperature ranges from 35.9 °C to 29.2 °C and the mean minimum temperature ranges between 24.5 °C and 19.8 °C (India Meteorological Department, 2015).

The *A. praemorsa* plants were growing as an epiphyte on *Guettarda speciosa* L. (Fig. 1A). The different types of roots collected included two types of aerial roots that were free of any substrate and were either positively or negatively geotropic (Fig. 1 B, C). Similarly, roots that were attached to bark (Fig. 1D) or growing in soil (Fig. 1E) were also collected. Ten cm roots were collected from three individuals. The roots were preserved in formalin-acetic acid-alcohol mixture (9 parts 70% ethyl alcohol, 0.5 parts 40% formalin and 0.5 parts glacial acetic acid). Transverse sections (TS) of unembedded roots were made 5 cm away from the tip using a razor blade. Sections that were ∼50–60 μm thick were stained with safranin, mounted on microscopic slides in glycerol and observed through an Olympus BX 51 bright field microscope. Images were captured using ProgRes C3 digital camera.

For assessing the mycorrhizal presence, the sections were immersed in 2.5% KOH for 2 h, at room temperature (27 ± 3 °C), washed in several changes of water, acidified with 5N HCl and stained with 0.05% trypan blue in lactoglycerol. The intensity of colonization was estimated on five randomly selected points in the root sections as the proportion of cortical cells containing fungal pelotons to the number of cells present in a microscopic field at ×200. The cell dimensions were measured using a calibrated ocular scale. Lamellar suberin was detected using Sudan IV, lignin and tannin were detected using Toluidine blue/HCl–phloroglucinol (Johansen, 1940; O'Brien et al., 1965), and Sudan Black was used for localizing lipids (Johansen, 1940).

For measuring root hair characteristics, 1-mm thick root sections were floated in the water on a microscopic slide. The number of root hairs was counted on 25 randomly selected root sections. The length of 25 randomly selected root hairs was measured using a calibrated ocular scale.

The anatomical terminologies used are as per Carlsward et al. (2006). Nevertheless, we used the term 'ectovelamen' instead of 'epivelamen' for the outer layer of the velamen as suggested by Pridgeon (2014).

Two-way analysis of variance (ANOVA) was used to assess the influence of substrate or root type on cell dimensions. One-way ANOVA or paired *t*-test was used to determine the variations in cell dimensions of different types of cells and the means were separated using Duncan's multiple range test (DMRT, for ANOVA). Linear regression analysis was used to examine the relationship between cell and peloton dimensions.

3. Results

3.1. Positively geotropic aerial roots

Velamen 3–5 cells wide. Ectovelamen uniseriate, cells primarily isodiametric with prominent ridge-shaped thickening (Fig. 2A,B). Endovelamen 2–4 cells wide with cells angular and radially elongate cells. Wall thickening of endovelamen cells more prominent than the cells of the ectovelamen. Ridged thickening more prominent in cells of the innermost layer of the endovelamen. Cover cells present over the short cells of the exodermis. Exodermis heterogeneous. Long cells possess typical ∩-shaped thickening. Cortex parenchymatous, irregular, with intrusions, 29–31 cells wide (Fig. 2C). Water storage cells with pitted walls present (Fig. 2D). Exodermis with different sized cells possessing O-shaped thickening (Fig. 2E). Smaller cells of the endodermis have extremely thickened walls than the larger cells. Pericycle cells thin-walled adjacent to the xylem and thick walled adjacent to the phloem. Vascular cylinder 24–33 arched. Xylem and phloem embedded in the sclerenchyma. Cell walls of the embedding tissue thickest around the phloem. Pith cells circular, thick walled enclosing triangular intercellular spaces. Lateral roots originate from the pericycle (Fig. 2F).

3.2. Negatively geotropic aerial roots

Velamen 4–5 cells wide (Fig. 2G). Ectovelamen cells large, isodiametric with prominently ridged thickenings. Endovelamen cells