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ROOT ANATOMICAL STUDIES IN TEN EPIPHYTIC ORCHIDS FROM WESTERN GHATS, GOA

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Abstract

Presently, an attempt was made to study the root anatomy in 10 selected epiphytic orchids [(*Acampe praemorsa* (Roxb.) Blatt. & McCann, *Aerides crispa* Lindl., *A. maculosa* Lindl., *Bulbophyllum sterile* (Lam.) Suresh, *Cottonia peduncularis* (Lindl.) Rchb.f, *Dendrobium barbatulum* Lindl., *D. macrostachyum* Lindl., *D. ovatum* (L.) Kranzl., *D. peguanum* Lindl., and *Rhynchostylis retusa* (L.) Blume] distributed in Western Ghats, Goa. The aerial roots of the selected species showed distinct variations in their internal organization. The components forming roots consisted of velamen, exodermis with the passage cells, cortex, endodermis, vascular bundles, and pith. Specific characters such as chloroplast, spiral thickening, needle-shaped crystals, and raphides were observed in the selected epiphytic orchid species.

Introduction

ORCHIDACEAE IS one of the largest and most diverse families in angiosperms, with about 705 genera and 29,481 species (POWO, 2023). These plants are the most pampered and occupy the top position amongst the flowering plants. Western Ghats form a large belt that receives high rainfall and shows different types of vegetation. In the Western Ghats, epiphytic orchids constitute the major group and show great variation in their morphological features. Orchids are widely distributed and occur mainly in the tropical, subtropical, and temperate regions (Prakash and Pathak, 2019). Taxonomically, they represent the most highly evolved family amongst monocotyledons. Epiphytic orchids are the most fascinating group. Their habitat is mainly at the top of giant tropical trees. They have modified aerial roots, clasping roots, pseudobulbs, and succulent leaves. The aerial hanging roots of epiphytic orchids possess a unique outer layer, the velamen, which is white or greyish in colour and consists of one to several layers of non-living cells. Epiphytic orchids show adaptations to the diverse ecological and climatic conditions (De and Biswas, 2022; Marwah *et al.*, 2021; Zotz and Winkler, 2013). Earlier studies by Moreira and Isaias (2008) have revealed the anatomical details of roots of epiphytic and terrestrial orchids from Brazil. The role of velamen in orchids has been reported earlier (Joca *et al.*, 2017; Zotz and Winkler, 2013) .Moreira *et al.* (2013) have stressed on the role of exodermis in epiphytic orchids. Senthilkumar *et al.* (2000b) uncovered the role of passage cells in the exodermis. In Goa, the distribution of orchids is mainly concentrated in the Western Ghats region, which accounts for 86% of the total orchid diversity of the state. There are about 67 species of orchids in 29 genera documented from Goa (Singh *et al.*, 2019). The present research is an attempt to study the root anatomy in selected 10 epiphytic orchids from Western Ghats, Goa.

Material and Methods

Study Area

Geographically, the State of Goa is located along the mid-west coast of India. It covers an area of about 3,702 sq.km from North to South. The state is sub-divided into two districts, North and South Goa district. South Goa district lies in the Southern part of Goa state. The geographical area of the district is 1,966 sq.km, and is situated between North latitudes 14°54' 00" and 15°29' 30" and between East longitudes 73°47' 00" and 74°20' 30". The district is bounded by Tiswadi, Ponda, and Satari talukas of North Goa district in the North, Uttar Kannada district of Karnataka in the East and South, and in the West by the Arabian Sea. The present study was carried out at various localities from Goa *i.e.* Dabhal, Dharbandora, Rivona, Tilamol, Quepem, Colomb, Netravali, Balli, Gaondongrem-Canacona, and Canacona.

Sampling

During the present study, 10 epiphytic species were selected from various localities of Goa *i.e.* Dabhal, Dharbandora, Rivona, Tilamol, Quepem, Colomb, Netravali, Balli, Gaondongrem-Canacona, and Canacona. The selected epiphytic species were *Acampe praemorsa* (Roxb.) Blatt. & McCann, *Aerides crispa* Lindl., *A. maculosa* Lindl., *Bulbophyllum sterile* (Lam.) Suresh, *Cottonia peduncularis* (Lindl.) Rchb.f,

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Dendrobium barbatulum Lindl., *D. macrostachyum* Lindl., *D. ovatum* (L.) Kranzl., *D. peguanum* Lindl., and *Rhynchostylis retusa* (L.) Blume. Selected orchids were collected in polythene bags and brought to the laboratory. Wet preservation was done by preserving in 70% alcohol. After a critical study of fresh floral material under dissection microscope, the selected species were identified with the help of local floras (Abraham and Vatsala, 1981; Rao, 1986) (Table 1).

Anatomical Studies

For anatomical studies, aerial hanging roots of 10 species of epiphytic orchids *i.e. Acampe praemorsa, Aerides crispa*, *A. maculosa*, *Bulbophyllum sterile, Cottonia peduncularis, Dendrobium barbatulum*, *D. macrostachyum, D. ovatum*, *D. peguanum,* and *Rhynchostylis retusa* were used. Thin free-hand sections were stained with dilute safranin and were observed under microscope for studying the internal details. Photographs of the sections were taken using a digital camera of Samsung attached to the microscope.

Mycorrhizal Studies

For mycorrhizal observation, the roots were sectioned just above the root tip, stained with 0.05% trypan blue (Phillips and Hayman, 1970) and were observed under microscope for hyphal and peloton formation.

Results

The present study revealed distinct anatomical variations among the studied epiphytic orchids. The results on the locality and flowering period of the studied orchid species are presented in Table 1 and the results on anatomical variations are depicted in Table 2 and Figs. 1-4.

The anatomical organization of roots consisted of velamen, exodermis with the passage cells, cortex, endodermis, vascular bundles, and pith. Specific characters such as chloroplast, spiral thickening, needle-shaped crystals, and raphides were also observed (Table 2).

Root Anatomy

1. *Acampe praemorsa* (Roxb.) Blatt. & McCann (Fig. 1a-f)

The anatomical characters showed that the velamen was 3-4 layered with cell wall thickening and polygonal shaped cells. Presence of epivelamen (outward extension of velamen cells) was also observed in the species (Table 2). The exodermis was single-layered with passage cells. Passage cells were observed as smaller than exodermal cells, and were located between exodermal cells. The exodermis had ∩ wall cell thickening. The cortex was many-layered with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of spiral thickening in the cortical cells and supra endodermal cells above the endodermis. Mycorrhizal fungi were present in cortical cells. Needle shaped

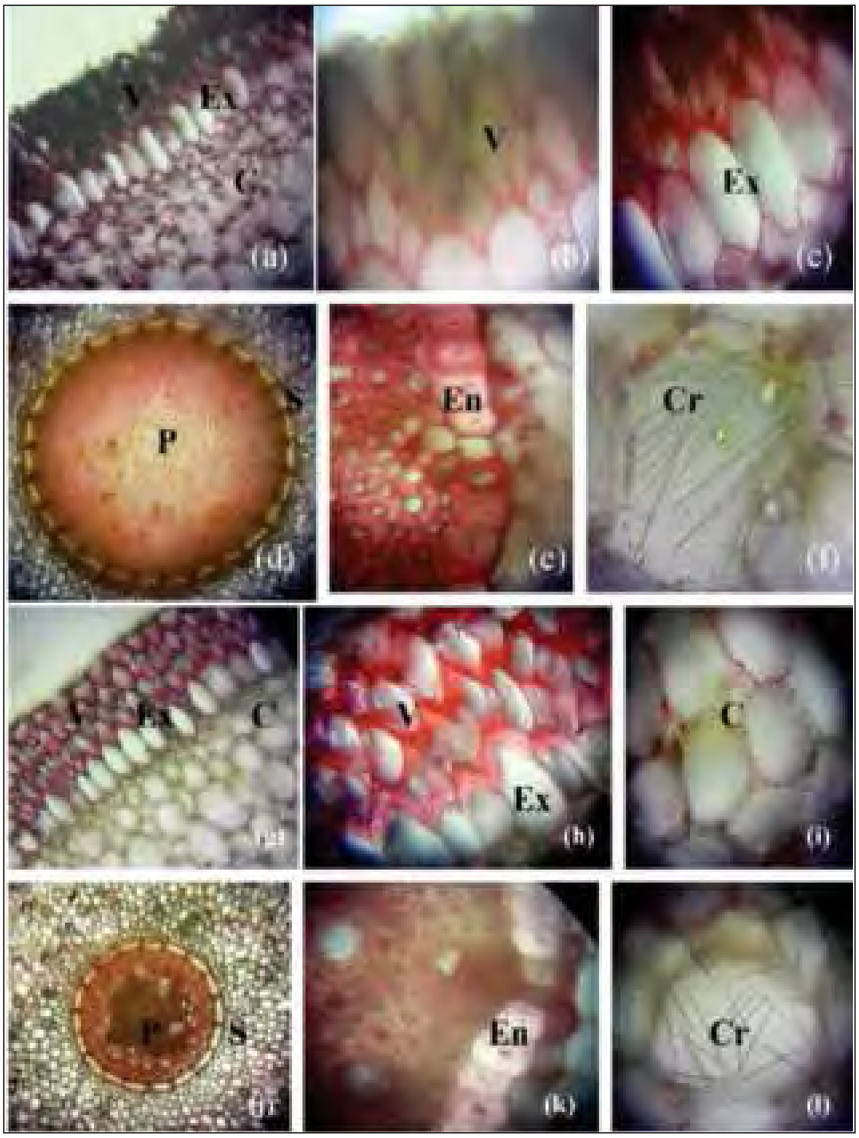

Fig. 1. Root anatomical studies in epiphytic orchids from Western Ghats, Goa: a-f, *Acampe praemorsa*; g-l, *Aerides crispa*. (V, Velamen; Ex, Exodermis; C, Cortex; S, Stele; En, Endoderrmis; Cr, Crystals; P, Pith).

Table 1. Occurrence and distribution of epiphytic orchids from Western Ghats, Goa.

Species	Host	Flowering period	Locality
Acampe praemorsa (Roxb.) Blatt. & McCann	*Mangifera indica, Carea arborea*	March-June	Dabhal, Quepem, Tilamol
Aerides crispa Lindl.	*Mangifera indica, Tamarindus indica*	May-June	Balli, Dabhal, Dharbandora, Canacona, Rivona
A. maculosa Lindl.	*Mangifera indica, Artocapus heterophyllus*	May-June	Dabhal, Dharbandora, Quepem, Canacona, Rivona, Colomb
Bulbophyllum sterile (Lam.) Suresh	*Anacardium occidental, Areca catechu*	Dec-January	Rivona, Colomb, Dabhal (Dharbandora), Netravali
Cottonia peduncularis (Lindl.) Rchb.f	*Mangifera indica, Artocapus heterophyllus*	March-May	Dharbandora, Gaondongrem-Canacona, Rivona, Colomb, Dabhal
Dendrobium barbatulum Lindl.	*Artocapus heterophyllus*	April-May	Gaondongrem-Canacona, Rivona
D. macrostachyum Lindl.	*Artocapus heterophyllus*	May-June	Gaondongrem-Canacona
D. ovatum (L.) Kranzl.	*Carea arborea, Mangifera indica, Artocarpus heterophyllus*	December-March	Netravali, Rivona, Quepem
D. peguanum Lindl.	*Anacardium occidentale*	December-January	Dharbandora
Rhynchostylis retusa (L.) Blume	*Mangifera indica, Anacardium occidentale*	June-August	Balli, Quepem, Dharbandora, Rivona

crystals were observed in the cortical region. Endodermis was single layered with O thickening.

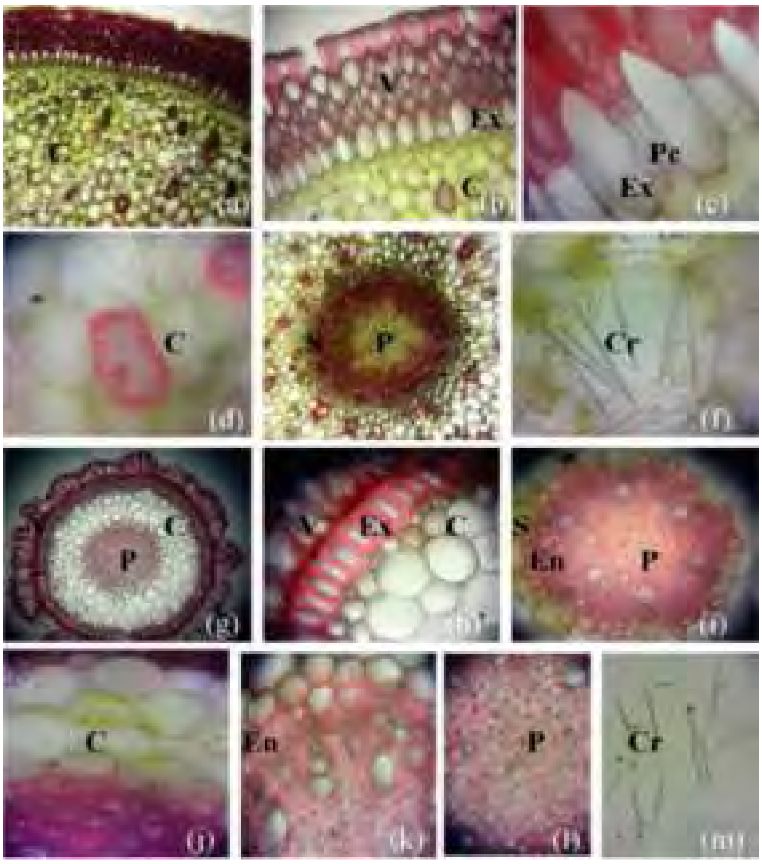

Fig. 2. Root anatomical studies in epiphytic orchids from Western Ghats, Goa: a-f, *Aerides maculosa*; g-m, *Bulbophyllum sterile.* (V, Velamen; Ex, Exodermis; C, Cortex; S, Stele; En-Endodermis; P, Pith; Cr, Crystals; Pc, Passage cell).

2. *Aerides crispa* Lindl. (Fig. 1g-l)

The anatomical characters revealed the presence of 2-3 layered velamen with cell wall thickening and polygonal shaped cells. Presence of epivelamen (outward extension of velamen cells) was also observed (Table 2). The exodermis was single-layered with passage cells. Passage cells were smaller than exodermal cells, and were located between exodermal cells. The exodermis had ∩ cell wall thickening. Cortex many-layered with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of chloroplast and spiral thickening, mycorrhizal fungi, and needle-shaped crystals. Endodermis was single-layered with O thickening.

3. *Aerides maculosa* Lindl. (Fig. 2a-f)

The anatomical characters revealed that the velamen was 3-4 layered with cell wall thickening and polygonal/hexagonal shaped cells. Presence of epivelamen (outward extension of velamen cells) was also observed (Table 2). The exodermis was single-layered with passage cells. Passage cells were much smaller than exodermal cells and were located between exodermal cells. The cortex was many-layered with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of chloroplast and spiral thickening, mycorrhizal fungi, and needle-shaped crystals. Endodermis was single layered.

4. *Bulbophyllum sterile* (Lam.) Suresh (Fig. 2g-m)

The anatomical characters showed irregular outline with single-layered velamen comprising elongated cells. The exodermis was single-layered, with O thickening (Table 2). The cortex had 4-5 layers with rounded-shaped cells. There were specific characteristics observed in the root section such as the presence of spiral thickening, mycorrhizal fungi, chloroplast, and needle-shaped crystals. Endodermis was single layered with O thickening. Pith was formed of thick-walled sclerenchymatous cells.

5. *Cottonia peduncularis* (Lindl.) Rchb.f. (Fig. 3a-f)

The root section was circular in outline with outer epivelamen layer. Next to epivelamen was 4-5 layered velamen with distinct cell wall thickening and polygonal-shaped cells (Table 2). The exodermis was single-layered, cells elongated, and polygonal/hexagonal with passage cells. Passage cells were smaller than exodermal cells and were located between exodermal cells. The exodermis had wall thickening on the outer side only. The cortex was many-layered with rounded to polygonal-shaped cells. Specific characteristics such as spiral thickening, chloroplast, mycorrhizal fungi, and needle-shaped crystals were observed in the root section. Endodermis was one-celled in thickness with O thickening of the wall.

6. *Dendrobium barbatulum* Lindl. (Fig. 3g-k)

The root section was circular in outline with outer epivelamen layer. Next to epivelamen was 4-5 layered velamen without any wall thickening, velamen cells isodiametric/hexagonal shaped (Table 2). The exodermis was a single-layered with passage cells. Passage cells were smaller than exodermal cells, and were located between ectodermal cells. Exodermis without cell wall thickening. The cortex had 4-5 layers with rounded to polygonal-shaped cells. Specific characters such as spiral thickening, chloroplast, mycorrhizal fungi, and needle shaped crystals were observed in the root section. Endodermis was single layered with no wall thickening.

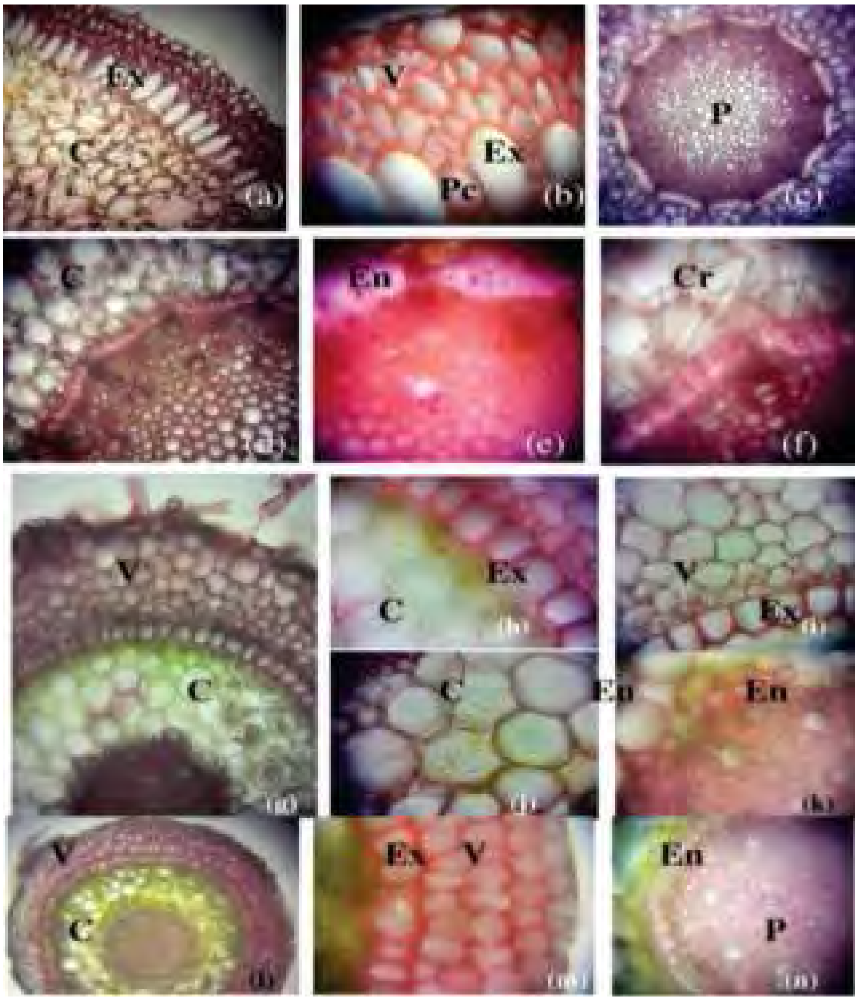

Fig. 3. Root anatomical studies in epiphytic orchids from Western Ghats, Goa: a-f, *Cottonia peduncularis*; g-k, *Dendrobium barbatulum*; l-n, *Dendrobium macrostachyum.* (V, Velamen; Ex, Exodermis; C, Cortex; En, Endodermis; P, Pith; Cr, Crystals; Pc, Passage cell).

7. *Dendrobium macrostachyum* Lindl. (Fig. 3l-n)

The root section was circular in outline with outer epivelamen layer and showed the presence of 2-3 layered velamen with hexagonal/polygonal shaped cells and with wall thickening (Table 2). The exodermis was single-layered with passage cells. Passage cells were smaller than exodermal cells, and were located between exodermal cells. The exodermis had an O wall thickening. Cortex 4-5 layered with rounded to polygonal shaped cells. There were specific characteristics such as the presence of spiral thickening and chloroplast in the cortical cells; mycorrhizal fungi, and needle-shaped were also observed. Endodermis was single-layered with thickening on the wall.

8. *Dendrobium ovatum* (L.) Kranzl (Fig. 4a-d)

The transverse section showed a circular outline with outer epivelamen layer. Next to the epivelamen layer was 5-6 layered

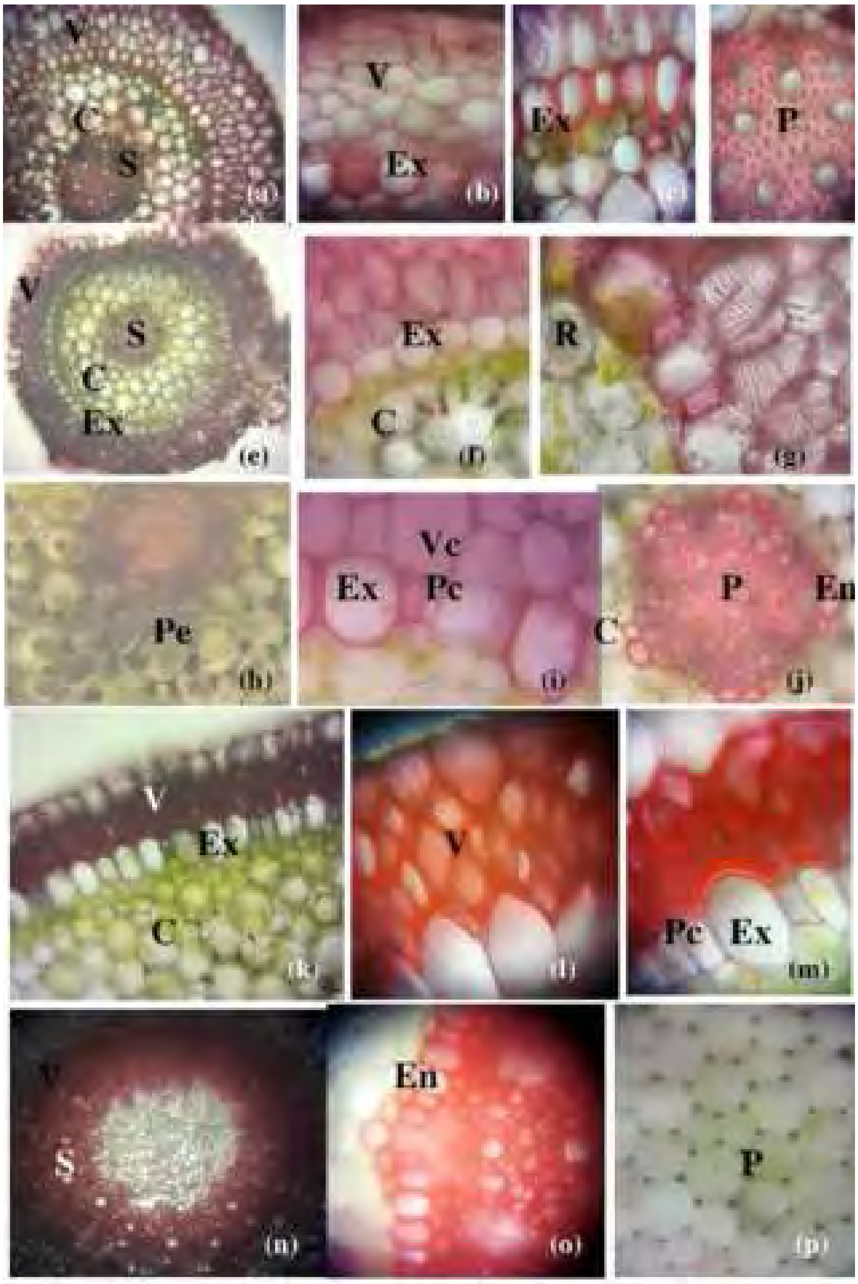

Fig. 4. Root anatomical studies in epiphytic orchids from Western Ghats, Goa: a-d, *Dendrobium ovatum*; e-j, *Dendrobium peguanum*; k-p, *Rhynchostylis retusa.* (Vc, Velamen cell; Ex, Exodermis; C, Cortex; S, Stele; En, Endodermis; P, Pith; R, Raphides; Pc, Passage cells).

velamen with wall thickening, velamen cells were isodiametric/hexagonal shaped (Table 2). The exodermis was single-layered with passage cells. Passage cells were smaller than exodermal cells, and were located between exodermal cells. Exodermis had O thickening. The cortex had 4-5 layers with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of spiral thickening, chloroplast, mycorrhizal fungi, and needle-shaped crystals. Endodermis was single layered with O thickening.

9. *Dendrobium peguanum* Lindl. (Fig. 4e-j)

The transverse section of root was circular in outline with a distinct epivelamen layer followed by 3-4 layered velamen with no wall thickening; velamen cells were isodiametric/ hexagonal shaped (Table 3). The cells of exodermis were thin-walled, single-layered interrupted by passage cells. The cortical region was 5-6 layered in thickness with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of spiral thickening, chloroplast, mycorrhizal fungi, raphides, and needle-shaped crystals. Endodermis was single layered with O thickening and passage cells.

10. *Rhynchostylis retusa* (L.) Blume (Fig. 4k-p)

The velamen was 3-4 layered with cell wall thickening and polygonal shaped cells. Presence of epivelamen (outward extension of velamen cells) was also observed (Table 2). The exodermis was single-layered with passage cells. Passage cells were smaller than exodermal cells, and were located between exodermal cells. The exodermis had ∩ wall cell thickening. The cortex was many layered with rounded to polygonal-shaped cells. There were specific characteristics such as the presence of spiral thickening, chloroplast, mycorrhizal fungi, and needle-shaped crystals in the cortical region. Endodermis was single-layered with O thickening and showed the presence of passage cells.

The present study revealed the presence of velamen tissues in the root section of all the studied species with the number of layers ranging from 1-6. The velamen layer was single-layered in *Bulbophyllum sterile* and was 5-6 layered in *Denbrobium ovatum.* The cells of velamen region were compactly arranged. In most of the studies species, the exodermis was thick-walled lignified with the exception of *Dendrobium barbatulum* and *D. peguanum* where the exodermal cells were formed of thin-walled cells. The cortical region ranged from a few to many layers and comprised of compactly to loosely arranged cells with varied cell size. In all the studied

Table 2. Root anatomy in 10 selected epiphytic orchids from Western Ghats, Goa.

Characters	*Acampe praemorsa*	*Aerides crispa*	*A. maculosa*	*Bulbophyllum sterile*	*Cottonia peduncularis*	*Dendrobium barbatulum*	*D. macrostachyum*	*D. ovatum*	*D. peguanum*	*Rhynchostylis retusa*
Supporting layer	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Velamen layers	3-4	2-3	3-4	1	4-5	4-5	2-3	5-6	3-4	3-4
Velamen cells	Polygonal/ Hexagonal	Polygonal	Polygonal/ Hexagonal	Elongated	Polygonal	Isodiametric/ Hexagonal	Hexagonal/ Polygonal	Isodiametric/ Hexagonal	Isodiametric/ Hexagonal	Polygonal
Velamen thickening	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes
Exodermis	Thickening not very distinct and seen only on outer side	Thickening only on outer side	Thin walled Round on outer side pointed towards inner side	Thickening O shaped	Thickening only on outer round wall. Round on outer side and pointed towards inner side	Thin walled Round on outer side pointed towards inner side	Round on outer side and pointed towards inner side	Round on outer side and pointed towards inner side	Thin walled Round on outer side pointed towards inner side	Round on outer side and pointed towards inner side
Exodermis layers	Single layered	Single layered thick walled	Single layered thin walled	Single layered thick walled	Single layered thick walled	Single layered thin walled	Single layered thick walled	Single layered thick walled	Single layered thin walled	Single layered thick walled
Exodermis thickening	Yes	Yes	Not distinct	Yes	Yes	No	Yes	Yes	No	Yes
Passage cells	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes
Cortex	Many layered	Many layered	Many layered	3-4 layered	Many layered	4-5 layered	4-5 layered	4-5 layered	4-5 layered	Many layered
Endodermis	Thick walled	Thick walled	Thick walled	Thick walled	Thick walled	Thin walled	Thin walled	Thick walled	Thick walled	Thick walled
Endodermis thickening	O	O	O	O	O	No	No	O	O	O
Passage cells	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Specific characters										
Chloroplast	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Spiral thickening	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mycorrhizal fungi	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Crystals	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped	Needle shaped

species, raphides and needle-shaped crystals were observed in the cortical region. The endodermal cells were uniformly thickened with lignified cell walls and were interrupted by passage cells. Vascular bundles varied in size. The difference was noted in the arrangement of vessels among the species. All the studied species exhibited the presence of pelotons in the velamen region, cortical region, and the hyphal penetration was also noted in the stellar region with hyphae penetrating through the passage of endodermal cells and making an entry into the pith region.

Discussion

In the present investigation, the root structure in all the 10 studied epiphytic orchid species was similar in its internal organization and comprised of epivelamen, velamen, cortex, and stele. The stele was monostelic in all the studied species with a central pith invaded by variable number of xylem and phloem strands, and endodermis with or without evident casparian strips. The number of cell layers, shape of the cell, and its distribution varied with the studied species. The variation in the number of cell layers could be attributed to the species and growing conditions (Benzing *et al.*, 1982; Dycus and Knudson, 1957; Ramudu and Khasim, 2018). Our observations in the present study are similar to the earlier findings made by Moreira and Isaias (2008) during their studies on comparative root anatomy of epiphytic and terrestrial orchids from Brazil. In the present study, the presence of velamen was observed in all the studied species. Literature studies reveal that many functions are attributed to velamen tissue, like mineral absorption, reducing transpiration loss, and providing mechanical protection (Pridgeon, 1986; Zotz and Winkler, 2013) and exchanging oxygen and carbon dioxide between the root and atmosphere (Sanford and Adanlawo, 1973). The velamen in orchid roots acts as a sponge, allowing the root to immobilize temporary but rightly accessible reservoirs of moisture and minerals (Benzing *et al.,* 1982; Hauber *et al*., 2020). Thus, velamen is an important adaptation of epiphytic orchids helping in water conservation and mechanical support (Zotz and Winkler, 2013). Exodermal cells which lies just below the velamen region provides mechanical protection and a high-humidity envelope around the cortex (Benzing *et al*., 1982; Moreira *et al*., 2013). In the present study, the roots in all the ten species showed thickening in endodermal cells, which could be the result of additional deposition of suberin and lignin (Fahn, 1990). The vascular cylinder exhibited similar characteristics in all the studied species with xylem and phloem strands and a central parenchymatic pith.

Mycorrhizal fungi which reside in the cortical cells are capable of ion uptake, once the velamen and the other exodermal cells are dead (Peterson and Enstone, 1996; Senthilkumar *et al*., 2000b). Orchid mycorrhizae are known for the translocation of sugars and phosphates to the orchid (Richardson *et al*., 1992). In the root transverse section, the pelotons appear as spherical and hexagonal balls in the parenchymatous cells of the cortical region (Bukhari and Gangaprasad, 2021). One of the important features of the orchid mycorrhiza is the lysis of the pelotons (Peterson and Currah, 1990). Mycorrhizal associations have already been reported in a few epiphytic orchids (Bhatti *et al.,* 2017; Lekshmi and Decruse, 2018; Manoharachary, 2019; Senthilkumar *et al*., 2000a). The mycorrhizal fungus helps in absorbing minerals and other nutrients from the surrounding medium and may probably be responsible for the supply of sugars to the orchid seed for the purpose of germination.

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