

RESEARCH ARTICLE

Vegetative anatomical adaptations of *Epidendrum radicans* (Epidendroideae, Orchidaceae) to epiphytic conditions of growth

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Received: 13.08.2017 | Accepted: 18.11.2017 | Published: 04.12.2017

Abstract

The anatomical properties of leaf, stem, and root of *Epidendrum radicans* Pav. ex Lindl., belonging to the subfamily Epidendroideae (Orchidaceae) were investigated for adaptations to stressed habitats. The anatomical investigation revealed that leaves of *E. radicans* have a thick cuticle (3–4 μm) and paracytic type of stomata. Foliar epidermal cells are conical on the adaxial surface and rectangular in the abaxial surface, distinct hypodermis absent, and uniseriate fiber bundles are arranged in both sides of the leaves. The foliar mesophyll is homogenous and starch grains and raphides present. The leaf sheath covering the stem have cuticle restricted to the outer surface and air spaces are present. The stem has a cuticularized uniseriate epidermis and a uniseriate hypodermis. The cortex and a parenchymatous ground tissue of the stem are separated by a layer of sclerenchymatous band. Vascular bundles are collateral and their size generally increases from the periphery towards the center. A sclerenchymatous patch covers the phloem pole, whereas the xylem is covered by thin-walled parenchymatous cells. The roots possess *Epidendrum*-type velamen. Cover cells present. Uniseriate dimorphic exodermis consists of U-thickened long cells and thin-walled passage cells. The endodermal cells O-thickened, pericycle sclerenchymatous, xylem 10–14 arched. The pith is sclerenchymatous, but parenchymatous at the center. The anatomical examination of *E. radicans* revealed adaptations to moisture stress conditions like thick cuticle covering the leaves and stem, water storage cells, multilayered velamen and dimorphic exodermis.

Keywords: *Epidendrum radicans*, cover cells, cuticle, idioblasts, raphides, starch grains, stegmata, velamen, water stress

Introduction

Epidendrum L. is the largest genus in the neotropical subtribe Laeliinae Benth. with around 1500 species and is native to the American continent (van den Berg *et al.* 2005; Pinheiro & Cozzolino 2013). The high diversity in *Epidendrum* is due to the different habitats they occupy ranging from dry tropical jungles to humid forests. In nature, most of these orchids grow as epiphytes, while some exist as lithophytes or terrestrial forms (Hágsater & Soto-Arenas 2005). Further, species of *Epidendrum* are also aggressive colonizers of the disturbed and denuded areas. *Epidendrum* taxa are proposed as a potential model for investigation on many evolutionary hypothesis including habitat selection and reproductive biology due to their wide variation in morphology, genetic diversity, ecological functionality and easy propagation (Pinheiro & Cozzolino 2013). The circumscription of *Epidendrum* is of great debate and several species that were once in this genus are now transferred to other genera (Chase *et al.* 2015). Economic importance of *Epidendrum* is limited when compared to other orchid genera. Stems of *E. mosenii* Rchb. f. are used as analgesic and *E. rigidum* Jacq. is reported to possess herbicidal activity. Infusion prepared from leaves of *E. chlorocorymbos* Schltr. is known to reduce blood cholesterol levels, cure spots on the skin, treat ear ache and stimulate dreaming (Asseleih *et al.* 2015). Nevertheless, species of *Epidendrum* are highly popular and most sought after by gardeners and hobbyists for their beautiful flowers and are popularly known as Poor man's orchid or Crucifix orchid (Stern & Carlsward 2009).

Anatomy helps in understanding the trends in plant evolution and adaptations although most of the recent concepts in these areas are based on molecular studies (Seago & Fernando 2013). Despite their huge species diversity and widespread popularity in horticulture, anatomical studies on species of *Epidendrum* are very limited. Baker (1972) noted that the lengths of guard cells in *E. parkinsonianum* Hook., ranged between 60 and 63 μm . Khasim & Mohana-Rao (1990) in their study on the

anatomy of some Epidendroideae reported the presence of fiber and compound midrib vascular bundles and the absence of trichomes, hypodermis and motor cells in leaves of *E. radicans* Pav. ex Lindl. In their comparative anatomy of the vegetative parts of Laeliinae, Stern & Carlsward (2009) examined the leaf anatomy of *E. anceps* Jacq. and *E. nocturnum* Jacq., among other members of the subtribe. The main foliar anatomical features noted were the absence of hairs, adaxial and abaxial (*E. anceps*) or abaxial (*E. nocturnum*) distribution of the stomata, reniform guard cells, lack of fibre bundles and hypodermis, undifferentiated mesophyll, and stigmata restricted to fibre bundles and vascular bundles. More recently, Moreira *et al.* (2013) compared the anatomical adaptations of the epiphytic *E. secundum* Jacq. and *Dichaea cogniauxiana* Schltr., growing in a nebular forest located at an amporupestre area at Serra da Piedade of Brazil to stresses of their microhabitats. In *E. secundum* the leaves growing in open light had more stomata and a thicker cuticle than *D. cogniauxiana* growing in the shade (Moreira *et al.* 2013).

Stem anatomy of *Epidendrum* is not well studied when compared to leaves and roots. Weltz (1897) examined the anatomy of stems in eight genera of Laeliinae and noted that the hypodermis in *Epidendrum* spp. was homogeneous, consisting of similar appearing cells. Investigations on the stem anatomy of *E. anceps* and *E. nocturnum* revealed the absence of hairs and stomata (Stern & Carlsward 2009). The cauline epidermis consisted of cells that was either thick- (*E. nocturnum*) or thin-walled (*E. anceps*), and the hypodermis contained cells with thickened walls. Vascular bundles were distributed in the ground tissue and the phloem and xylem were covered by crescent shaped sclerenchymatous caps. Stigmata often associated with the phloem sclerenchyma (Stern & Carlsward 2009).

Dycus & Knudson (1957) while examining the role of velamen in aerial roots of orchids observed that the number of velamen layers in *E. xanthium* Lindl., *E. boothianum* Lindl., *E. ibaguense* Lindl. and *E. radicans* ranged from 3 to 5. Pridgeon *et al.* (1982) examined

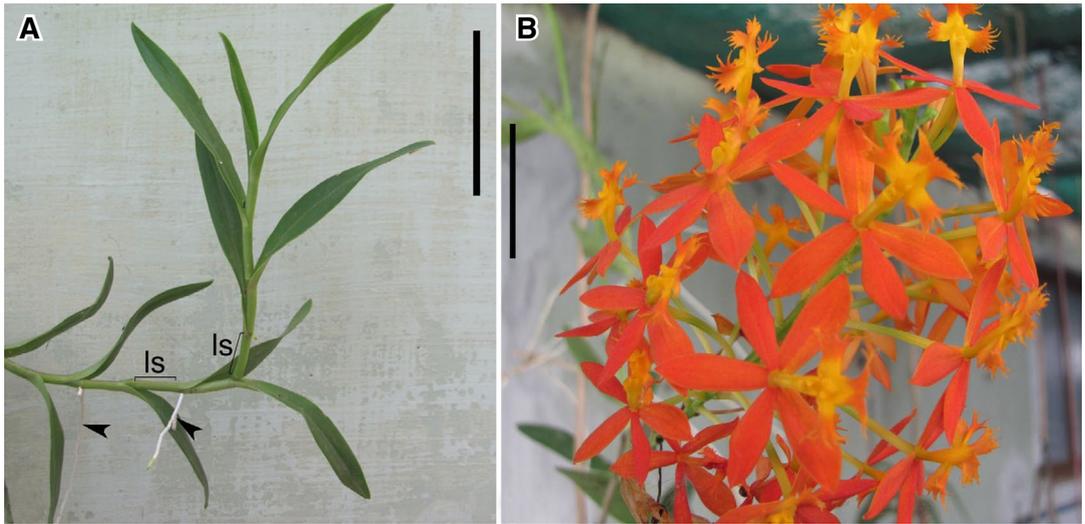


Fig. 1. Habit of *Epidendrum radicans*. **A** – vegetative shoot with roots (**arrow heads**) and the stem covered by leaf sheath (**ls**); **B** – flowering shoot. Scale bars = 5 cm (**A**) and 2 cm (**B**).

13 species of *Epidendrum* for the presence of tilosomes in roots. Of these, elaborate tilosomes were present only in *E. coriifolium* Lindl., and tilosomes of limited nature were observed in *E. ensatum* A. Rich. & Gal., *E. pseudepidendrum* (Rchb. f.) Rchb. f. and *E. secundum* Jacq. Moller & Rasmussen (1984) indicated the occurrence of conical silica structures in *Epidendrum*. Porembski & Barthlott (1988) in their classification of velamen radicum grouped orchids with velamen resembling *E. bifidum* Abul. as *Epidendrum*-type. Epivelamen cells in this velamen type are smaller than the radially elongated endovelamen cells. Further, endovelamen cells have thickenings that are fused into composed ledges and large pores. Tilosomes are infrequent and walls of the endodermis lightly thickened. Cortex may possess tracheoidal idioblasts (Porembski & Barthlott 1988). Zankowski *et al.* (1987) investigated the developmental anatomy of velamen and exodermis in aerial roots of *E. ibaguense* and concluded that casparian strips in the exodermis are obscure and are not a functional equivalent of endodermis. Oliveira & Sajo (1999) studied the root anatomy of *E. campestre* Lindl. and *E. secundum* growing at Instituto de Botânica de São Paulo. Root hairs were present in *E. campestre*. The velamen

in *E. campestre* was heterogeneous with periclinally flattened exovelamen and an endovelamen with finely reticulate thickened walls. Cover cells were present in both the species. The velamen consisted of 5–6 cell layers in both species, exodermal cells were O-thickened (*E. campestre*) or U-thickened (*E. secundum*), cortex was 9–12-layered, endodermis was O-thickened and the xylem arches ranged from 11 to 17. The root cortex of *E. campestre* had fungal hyphae and water storing idioblasts. Nevertheless, velamen cell layers, as well as the water storing idioblasts, in roots were observed in the both species (Oliveira & Sajo 1999). These anatomical modifications indicate adaptation of the two orchids to their microhabitats (Moreira *et al.* 2013). The anatomy of *E. anceps* and *E. nocturnum* roots was characterized by the lack of tilosomes and cortical cell wall banding, and exodermal cells with U-thickened walls. Endodermal and pericycle cells in these taxa were isodiametric with O-thickened walls (Stern & Carlsward 2009).

Among the different species of *Epidendrum*, the ground dwelling *E. radicans* (Fig. 1), commonly known as fire star orchid or ground root orchid, is indigenous to Central America (Devadas *et al.* 2010). In addition to terrestrial

habitats, *E. radicans* also thrives as epiphyte in the Asian tropics (Khasim & Mohana-Rao 1990). Unlike other orchids where flowering is usually seasonal, this orchid produces flowers year round (Janzen 1987; Suttleworth *et al.* 1994). *E. radicans* often occurs in anthropogenically disturbed areas like the roadsides at an altitude of 1,000–2,000 m a.s.l. (Bierzychudek 1981). Human activities are shown to increase the abundance of *E. radicans* (Dressler 1981). Epiphytic habitats are always stressful due to the lack of water and nutrient holding medium. Plants inhabiting these habitats have evolved several structural and physiological adaptations that enable these plants to successfully thrive in these circumstances. In spite of its weedy nature, *E. radicans* is susceptible to stresses and requires optimum moisture, light and nutrients for normal growth (Dressler 1981). Therefore, the aim of the present study was to examine the vegetative anatomy of *E. radicans* and to record the adaptations that help this species to survive in epiphytic and other stressful habitats.

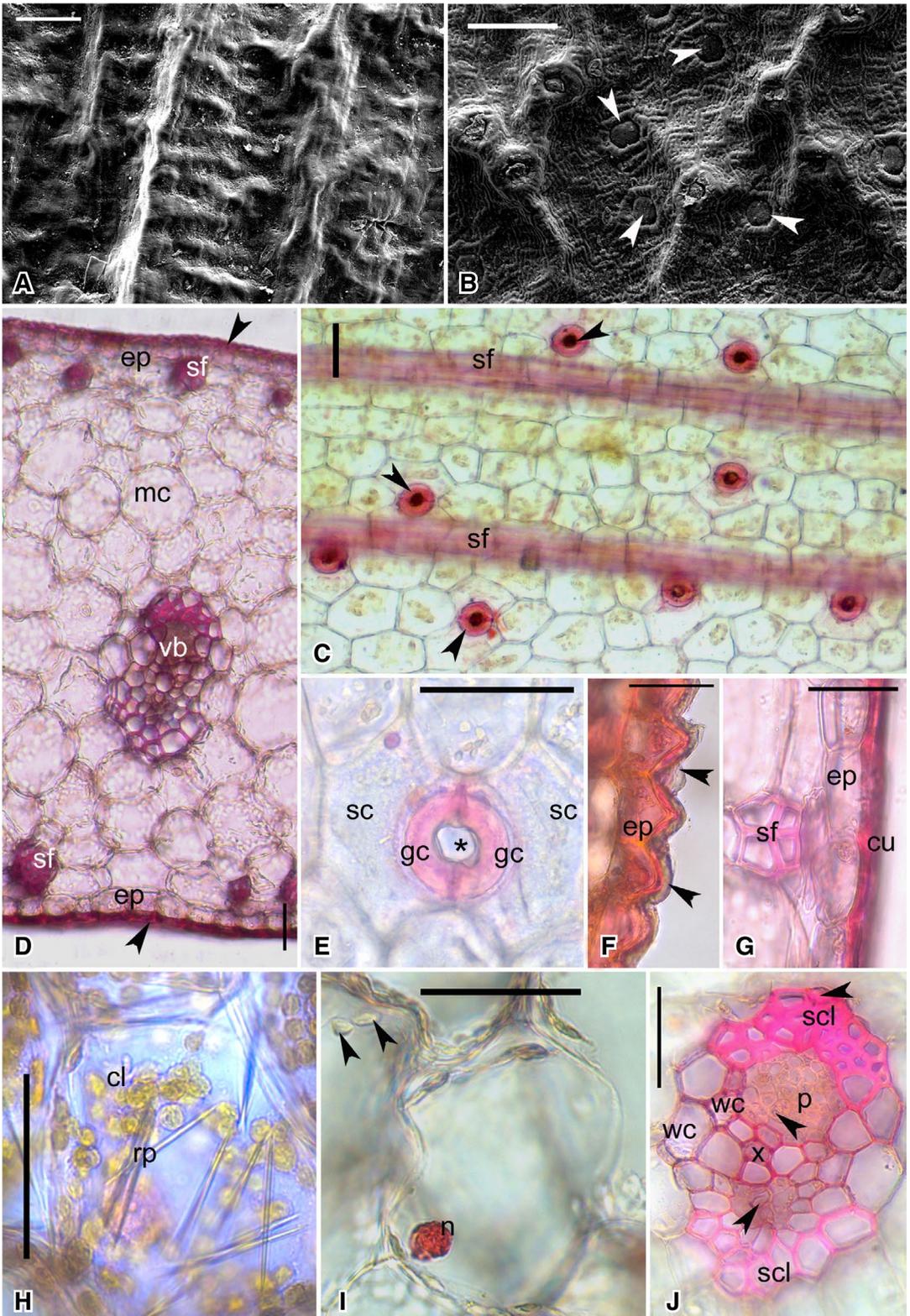
Material and methods

The vegetative material of *E. radicans* for anatomical studies was obtained from a home garden (10°59'54.2" N, 76°59'22.9" E, 411 m a.s.l.) in Coimbatore, Tamilnadu, India. The average maximum and minimum temperature of Coimbatore are 32.5°C and 21.3°C. The relative humidity ranges from 49–88%. The plants were growing in a light intensity of 27.87 candelas and in 18 cm clay orchid pots filled with charcoal, broken bricks and coconut husk in the ratio of 1 : 1 : 2. The plants were watered once the potting

medium dried and standard fertilizer was applied as foliar spray once every fifteen days. Plant samples from three potted plants were collected during January of 2017. Fresh stems, roots and leaves were collected, washed and fixed in FAA (formalin – acetic acid – alcohol) mixture until processing (Johansen 1940). For uniformity, fifth leaf from the tip was selected for examination and the sections were made midway between the tip and the base. Similarly, stem and root were sectioned 7.5 cm and 5 cm respectively from the tips. Free hand sections 30–40 µm thick were taken using a razor blade and stained with safranin. Lamellar suberin was tested by staining with sudan IV, toluidine blue / HCl-phloroglucinol was used to locate lignin and tannin, and iodine was used to detect the presence of starch (Johansen 1940).

The sections either stained or treated with various histochemical reagents were mounted in glycerine and observed under an Olympus BX51 light microscope. A calibrated ocular scale was used to measure the dimensions of the cells and the size of the different regions in the sections. The values are presented either as range or length [mean ± standard error (SE)] × width (mean ± SE) or mean ± SE accordingly. Microphotographs of the sections observed were captured with a ProgRes3 camera attached to the Olympus BX 51 microscope. Stomatal index (%) was calculated (n=10) from the number of epidermal cells and stomata in ten randomly selected microscopic fields (×200) according to Salisbury (1927). For scanning electron microscopy (SEM), 5 mm² of leaf bit or thin transverse section of stem and root were fixed with double-sided adhesive tape onto labelled stubs. The specimens were coated with gold and processed in Philips SEM 515.

Fig. 2. Leaf anatomy of *Epidendrum radicans*. **A–B** – scanning electron microscope images of the adaxial leaf surface showing ridges and groves (**A**) and abaxial surface with stomata (**white arrow heads**) (**B**); **C** – epidermal peeling of leaf showing stomata (**black arrow heads**) and sclerenchymatous fiber bundles (**sf**); **D** – transverse section (**t.s.**) of leaf showing cuticle (**black arrow heads**), epidermis (**ep**), sclerenchymatous fiber bundle (**sf**), homogenous mesophyll (**mc**) and collateral vascular bundle (**vb**); **E** – paracytic stomata (**asterisk**) on the abaxial leaf surface surrounded by guard cells (**gc**) and subsidiary cells (**sc**); **F** – t.s. of leaf showing adaxial epidermis (**ep**) with triangular cells and cuticle (**black arrow heads**); **G** – t.s. of leaf showing abaxial epidermis (**ep**) covered by a cuticle (**cu**) and the presence of sclerenchymatous fiber bundle (**sf**); **H** – t.s. of leaf showing mesophyll cells containing chloroplasts (**cl**) and raphides (**rp**); **I** – t.s. of leaf showing mesophyll cell containing intact nucleus (**n**) and starch grains (**black arrow heads**); **J** – t.s. of leaf showing vascular bundle with xylem (**x**), phloem (**p**), sclerenchymatous cap (**scl**), water cells (**wc**) and stegmata (**arrow heads**). Scale bars = 50 µm (**A–J**).



Results

Leaf

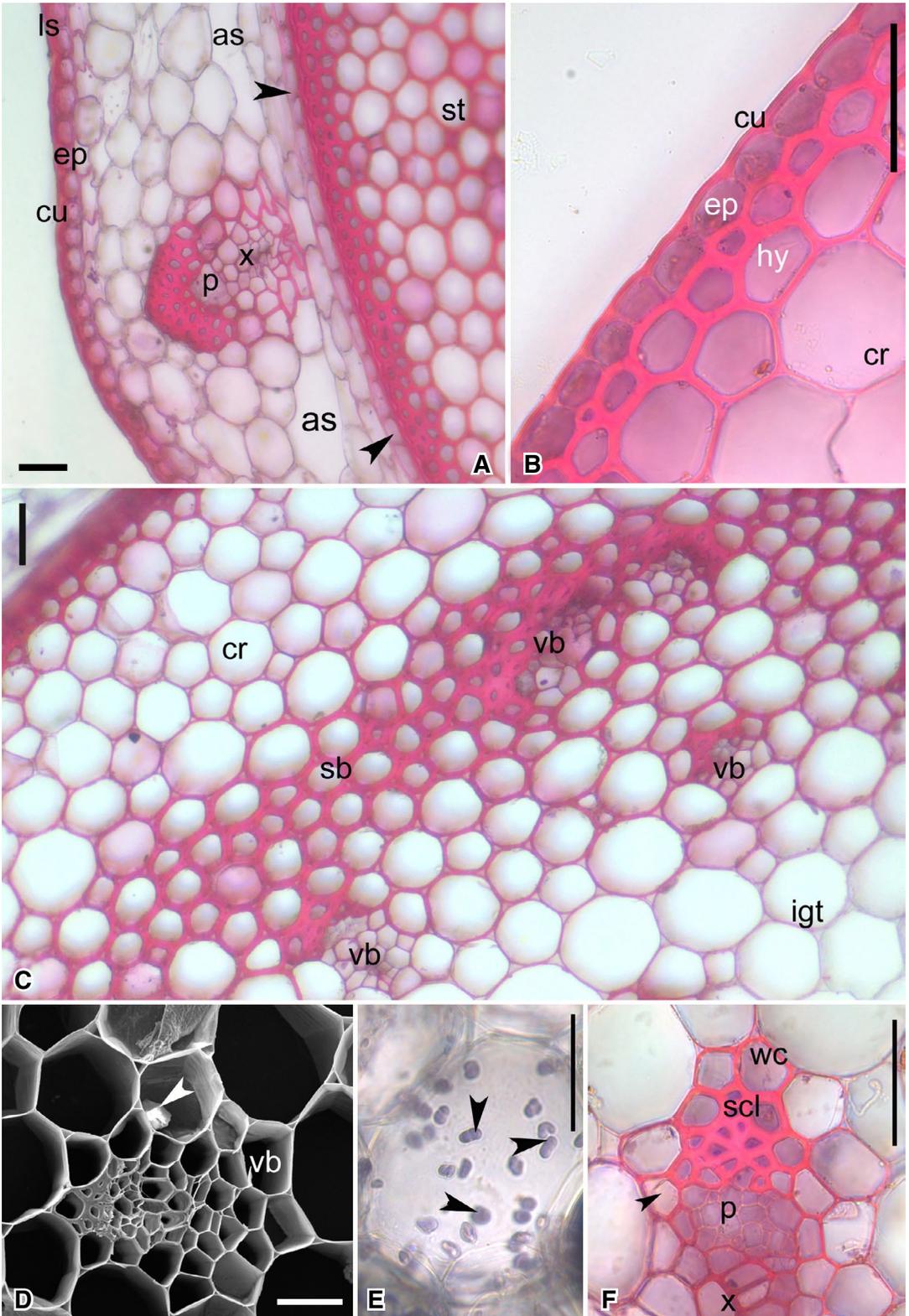
Cuticle 3–4 μm thick and smooth, present on both abaxial and adaxial surfaces of the leaf (Fig. 2 A, D, F, G). Paracytic stomata restricted to the adaxial surface, with two subsidiary cells having longitudinal axes parallel to the guard cells of the aperture (Fig. 2 B, C, E). Substomatal chambers small and irregularly shaped. The subsidiary cells fail to meet over the poles. The pore measures $14.05 \pm 0.18 \times 13.65 \pm 0.18 \mu\text{m}$ (length \times breadth). The guard cells measure $31.9 \pm 0.33 \times 12.6 \pm 0.28 \mu\text{m}$ and subsidiary cells measure $33.15 \pm 0.55 \times 54.25 \pm 0.69 \mu\text{m}$. The calculated stomatal index is $9.77 \pm 0.34\%$. Upper and lower epidermis uniseriate, compactly arranged, thick walled, nucleate and parenchymatous. Cells of the upper epidermis are conical and measure $25.57 \pm 0.55 \times 38.65 \pm 0.41 \mu\text{m}$, and those of the lower epidermis are rectangular and measure $33.00 \pm 0.54 \times 23.50 \pm 0.41 \mu\text{m}$. Hypodermis consists of thin walled parenchymatous cells. Fiber bundles present in the hypodermal region of both the upper and lower surfaces. However, the number of fiber bundles in the adaxial region is higher compared to that of the abaxial region. Mesophyll cells 23–25 μm (23.5 ± 0.17) wide, homogenous and not differentiated into palisade and spongy layers. Starch grains that stain brown with iodine are present in most of the mesophyll cells. Raphides present. Vascular bundles arranged in a single row in the median. Xylem and phloem are covered by a sclerenchymatous cap (Fig. 2 D, G, J). The sclerenchymatous cap covering the phloem is much bolder than those covering the xylem. Small water storing idioblasts present next to the phloem. Largest vascular bundle is present

in the midrib region. In the lamina, a large vascular bundle alternates 2–4 smaller vascular bundles. Stegmata present in xylem, phloem and sclerenchymatous cap surrounding the phloem (Fig. 2 J).

Stem

Stems circular, green, smooth, hairs absent, and the leaf sheath cover 50 % of the internode. Leaf sheath consist of circular to oval thick-walled epidermal cells covered by a 2–5 μm thick cuticle (Fig. 3 A–C). Epidermis and cuticle restricted to the adaxial surface of the leaf sheath. The epidermis is followed by a 7–8 layers of parenchymatous cells that are circular to irregular enclosing small triangular intercellular spaces. The innermost layer of the leaf sheath consists of 1–2 rows of rectangular cells with slightly thickened walls. Large airspaces present in the leaf sheaths. Leaf traces collateral with the phloem pole covered by a thick sclerenchymatous cap and the xylem pole covered by cells that are lightly thickened compared to the phloem pole. Cuticle covering the stem is 2–6 μm thick. Cuticle in the stem in regions covered by leaf sheath is thinner (2–4 μm) than those regions not covered by the leaf sheath (3–6 μm) (Fig. 3 A, B). Epidermis is uniseriate, with compactly arranged rectangular parenchymatous cells and measure $29.03 \pm 0.67 \times 20.07 \pm 0.45 \mu\text{m}$. Hypodermis consists of 1–3 layers of compactly arranged thick walled sclerenchymatous cells. Tissue inner to the hypodermis consists of 49–55 layers of cells and differentiated into an outer cortex and inner ground tissue regions. The cortex and the ground tissue are separated by 4–5 layered sclerenchymatous bands. Outer cortical region is 4–6 layered parenchymatous or sometimes chlorenchymatous enclosing triangular

Fig. 3. Leaf sheath and stem anatomy of *Epidendrum radicans*. **A** – transverse section of leaf sheath (**ls**) covering the stem (**st**), showing cuticle (**cu**), epidermis (**ep**), air spaces (**as**) and the inner layer of the leaf sheath (**black arrow heads**); **B** – stem transverse section showing cuticle (**cu**), epidermis (**ep**), hypodermis (**hy**) and cortex (**cr**); **C** – outer cortex (**cr**) and inner ground tissue (**igt**) separated by a sclerenchymatous band (**sb**) and vascular bundles (**vb**); **D** – SEM image of the inner ground tissue containing stegmata (**white arrow head**) and vascular bundle (**vb**); **E** – starch grains (**black arrow heads**) in the ground tissue cell; **F** – vascular bundle with xylem (**x**), phloem (**p**), sclerenchymatous cells (**scl**), water cell (**wc**) and stegmata (**black arrow head**). Scale bars = 50 μm (**A–F**).



intercellular spaces. Vascular bundles absent in the cortex. The inner ground tissue contains scattered vascular bundles with the outermost bundles immersed in the sclerenchymatous band. The size of the vascular bundles generally increases towards the centre of the stem. Starch grains are present in most of the ground tissue cells (Fig. 3 E). The vascular bundles are collateral and the phloem pole is covered by sclerenchymatous cells. Stegmata present both in the cortical and phloem regions (Fig. 3 D, F).

Root

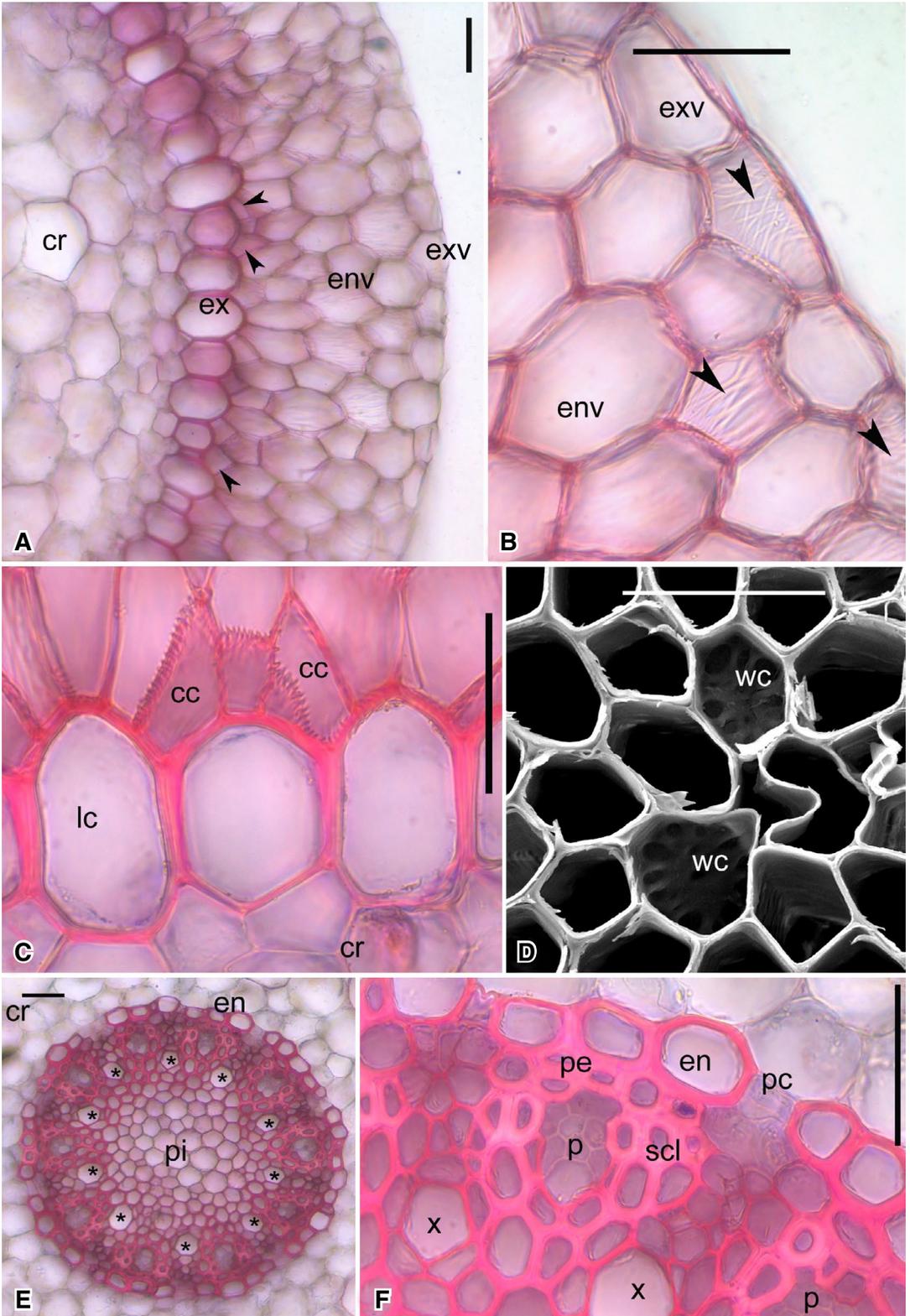
The aerial roots of *E. radicans* are circular, whereas those attached to the substratum have a flattened region at the point of contact with the substratum. The root is covered by a velamen that is 4–6-layered (Fig. 4 A, B). Velamen is differentiated into an outer exovelamen and an inner endovelamen. The exovelamen is uniseriate with isodiametric cells. The endovelamen is 3–5-layered consisting of isodiametric to radially elongated cells. Cells of the endovelamen are comparatively larger. Wall striations are present in the cells of both the exovelamen and endovelamen. The average velamen cell measures $63.35 \pm 1.01 \times 35.25 \pm 0.64 \mu\text{m}$. The velamen is followed by the exodermis, which is the outermost cortical layer. The exodermis consists of long and short cells. The long cells of the exodermis are U-thickened and the short passage cells are comparatively thin-walled. The wall of the long cells reacts positive to suberin. The exodermis cell measures $60.75 \pm 0.54 \times 35.45 \pm 0.84 \mu\text{m}$. Small cover cells are present above the exodermal layer. Below the exodermis is an 8–9-layered thin-walled parenchymatous cortex (Fig. 4 C–E). The cortical cells of the substrate roots contain fungal pelotons in root portions attached to the substrate. The ultimate cortical layer is

differentiated into uniseriate endodermis. The endodermal layer consists of cells with O-thickened walls that reacts positive to suberin, and is interspersed with 1–2 thin-walled passage cells (Fig. 4 F). Pericycle uniseriate, cells facing the xylem are thin-walled and those opposite to the phloem are thick-walled. Vascular cylinder 10–14-arched. Vascular tissue is surrounded by sclerenchymatous tissue. The pith is sclerenchymatous with thick-walled cells. But pith cells at the centre may be thin-walled enclosing small intercellular spaces. Water cells present in the cortical and pith region (Fig. 4 D).

Discussion

Generally, plants that thrive in extreme environmental conditions have adaptations that help them to overcome the different stresses. *E. radicans* is able to exist in open and rocky areas, which are strictly terrestrial, and sometimes grows as an epiphyte. Though *E. radicans* shows a tendency of weedy growth, the plant cannot tolerate stress and it needs adequate light, water and nutrients supplement (Dressler 1981). Anatomical adaptations are evident in all the vegetative parts of *E. radicans*. The leaves and stems lack hairs as reported for other *Epidendrum* species. This contradicts the studies where frequent occurrence of trichomes was reported in Laeliinae including *Epidendrum* species (Solereeder & Meyer 1930; Baker 1972). Stern & Carlsward (2009) also failed to detect trichomes on leaves of Laeliinae members including *E. anceps* and *E. nocturnum*. These authors suggested that the lack of trichomes in Laeliinae could be due to the fragile thin-walled nature of the terminal cells of the trichomes that detach off very easily. Nevertheless, examination of young unopened leaves of *E. radicans* also revealed the absence of trichomes suggesting

Fig. 4. Root anatomy of *Epidendrum radicans*. **A** – transverse section of root showing exovelamen (**exv**), endovelamen (**env**), cover cells (**black arrow heads**), exodermis (**ex**) and cortex (**cr**); **B** – striations (**black arrow heads**) in cells of exovelamen (**exv**) and endovelamen (**env**); **C** – exodermis with long cells (**lc**) and cover cells (**cc**); **D** – scanning electron microscopic image of water cells (**wc**) in the cortex; **E** – cortex (**cr**) and stele showing endodermis (**en**), xylem arches (**asterisks**) and pith (**pi**); **F** – endodermis (**en**) with passage cells (**pc**) and pericycle (**pe**) covering the phloem (**p**) and xylem (**x**) embedded in sclerenchymatous tissue (**scl**). Scale bars = 30 μm (**D**) and 50 μm (**A–C, E, F**).



that foliar trichomes were absent in this orchid. The leaves of *E. radicans* are hypostomatic like in other *Epidendrum* species. But stomata of *E. radicans* are not tetracytic as in other Laeliinae members (Khasim & Mohana-Rao 1990; Stern & Carlsward 2009), they are paracytic as reported by Khasim & Mohana-Rao (1990). The size of guard cells is well within the range reported for other members of Laeliinae (Stern & Carlsward 2009).

In this study, leaf sheath and stems of *E. radicans* were covered by a cuticle that varied in thickness. The thick cuticle is suggested as an adaptation to minimize water loss in epiphytic orchids (Moreira *et al.* 2013; Yang *et al.* 2016). The cuticle is also known to play a key role in the exchange of gases, it protects plants against environmental stresses and generates a suitable microenvironment for phyllosphere organisms (Yeats & Rose 2013; Fernández *et al.* 2016). Epidermal cells of *E. radicans* have an outer undulating anticlinal walls resulting in conical shaped cells similar to those reported in several desert plants (Gibson 1996). Nevertheless, the foliar epidermal cells on the abaxial side are periclinal. Though foliar hypodermis has been reported in several members of Laeliinae, it appear to be absent in *Epidendrum* species as observed in the current and other studies (Stern & Carlsward 2009). Foliar fibre bundles in *E. radicans* are distributed on both sides of the leaves resembling those of *E. nocturnum*. Nevertheless, the distribution of foliar bundles was mostly abaxial in most members of Laeliinae including *E. anceps* and appears to be the usual anatomical feature in this group (Stern & Carlsward 2009). Foliar fiber bundles are mechanical in function supporting the leaves and have no systematic value as they occur in many epiphytic orchid taxa across various groups (Stern & Carlsward 2009). The mesophyll of *E. radicans* is homogenous as in other species of *Epidendrum* (Khasim & Mohana-Rao 1990; Stern & Carlsward 2009). Unlike in *E. anceps* and *E. nocturnum*, where the vascular bundles occur in two rows, the vascular bundles in leaves of *E. radicans* are arranged in a single median row. The distribution of stegmata in vascular bundles is

similar to those of *E. anceps* and *E. nocturnum* (Stern & Carlsward 2009).

In *E. radicans*, leaf sheaths cover almost half of the internode of the stem and may provide mechanical support to the stem. To our knowledge anatomical features of the leaf sheath in *Epidendrum* or any other orchid species has never been examined before. The anatomy of leaf sheath is different from those of the leaves. The leaf sheaths are characterized by distinctive anatomical characters like the presence of large air spaces, the absence of cuticle on the abaxial surface, stegmata and idioblasts. Vascular bundles occupy nearly two third width of the leaf sheath. The sclerenchymatous patch covering the phloem is more prominent than those covering the xylem.

Idioblasts with different types of thickenings were found in all vegetative organs of *E. radicans*. It is believed that idioblasts can assist in the storage of water (Pridgeon 1982) and are termed as water cells by Stern & Carlsward (2009). However, these specialized cells can also afford mechanical support by preventing cell collapse during water stress and appears to be an adaptation to xeromorphic conditions of the epiphytic habitats (Olatunji & Nengim 1980; Holtzmeier *et al.* 1998). According to Pridgeon (1982), during the shortage of water, these cells either get filled with air or may become involved in water storage.

The stem anatomy of *E. radicans* resembles in general other members of Laeliinae (Stern & Carlsward 2009). The stem of *E. radicans* is devoid of hairs and stomata, and the thickness of the cuticle covering the stem is well within the range reported for other members of Laeliinae (Stern & Carlsward 2009). The variation in the thickness of the stem cuticle in the leaf sheath covered and uncovered portion clearly suggests the role of leaf sheath in minimizing water loss in additions to aiding rigidity to the stem. The epidermal cells in *E. radicans* are conical similarly to those of *E. nocturnum* and thin-walled (Stern & Carlsward 2009). Though most members of Laeliinae lack a cortex, *E. radicans* has a cortex that is 4–6 cells wide. A fibre band in *E. radicans* covers the ground tissue, as in *Broughtonia* R. Br. (Stern & Carlsward 2009).

The ground tissue in *E. radicans* consists of both large and small roundish cells unlike in *Orleanesia* Barb. Rodr., where the cells of the ground tissue are more or less evenly sized (Stern & Carlsward 2009). Though cauline stigmata were reported in stems of *E. anceps* and *E. nocturnum* by Stern & Carlsward (2009), Khasim & Mohana-Rao (1990) did not observe any stigmata in stems of *E. radicans*. In contrast, stigmata were observed in cells adjoining the vascular bundles in *E. radicans* in the present study. Moller & Rasmussen (1984) suggested that silicon enters epiphytic plants mainly due to the settling of the air-borne dust or through the water run-offs containing silica from the phorophytes. Further, the widespread presence of stigmata in xerophytes and their absence in mesophytes suggests the possibility of the relation of stigmata to arid conditions (Moller & Rasmussen 1984).

The aerial roots of *E. radicans* are cylindrical whereas the roots attached to the substratum are flattened at the point of contact and the former lacked root hairs. This is similar to the observations of Stern & Carlsward (2009) and Moreira *et al.* (2013) where root hairs were absent in aerial roots of studied *Epidendrum* species. In contrast, root hairs were present in root regions that were in contact with the substratum as in *E. secundum* (Moreira *et al.* 2013). The velamen in *E. radicans* resembles the velamen in other members of Laeliinae and is of typical *Epidendrum*-type. The velamen is an important structure in orchid roots adapted for the uptake of water and dissolved nutrients. It helps in the quick absorption of water and prevents moisture loss from the roots, apart from providing mechanical protection, reflecting infra-red radiation, screening of roots against ultraviolet radiation and absorbing immobilized nutrients from rain water (Thangavelu & Ayyasamy 2017). Apart from this, the other functions that can be attributed to this tissue include amplifying access to mineral-rich solutions (Benzing *et al.* 1982), and exchange of carbon dioxide and oxygen between the root and atmosphere (Moreira & Isaias 2008).

The occurrence of velamen is generally associated with an epiphytic habit (Zotz

& Winkler 2013), though it occurs also in terrestrial orchids (Porembski & Barthlott 1988; Uma *et al.* 2015). The size of the velamen can be related to specific environmental factors like temperature and water. Hence, orchids like *E. radicans* occurring in dry or exposed habitats have a multilayered velamen, while those occurring in humid environments are characterized by few-layered velamen (Sanford & Adanlawo 1973). Moreover, the velamen in *E. radicans* is differentiated in two layers, the exovelamen being uniseriate and the endovelamen – multiseriate. On the other hand, wall striations were observed in the cells of both exovelamen and endovelamen in *E. radicans*. The striations in the exovelamen of *E. radicans* falls into the type-IIIa and those of the endovelamen falls into the type-IIb according to Sanford & Adanlawo (1973) classification. The wall thickenings in the velamen provide mechanical support to the cells avoiding their collapse during the dehydration (Noel 1974; Oliveira & Sajo 1999).

Exodermis, the outer layer of the cortex (Engard 1944) in *E. radicans* is heterogeneous consisting of long and short cells. The long cells of the exodermis develop secondary thickenings in their walls during maturity and die (Pridgeon 1986). The high lignin and suberin content in the walls of the exodermal long cells provide mechanical strength and maintains high humidity around the cortex (Sanford & Adanlawo 1973; Benzing *et al.* 1983; Moreira & Isaias 2008). The shorter passage cells are thin-walled and living, they play a significant role in the nutrition and hydration as in other epiphytic orchids. The 2–4 wedge-shaped cover cells above the short cells of the exodermis as seen in *E. radicans* are formed from the innermost layer of the velamen (Carlsward *et al.* 2006). Though cover cells are associated with tilosomes, in some orchids we did not observe any tilosomes originating from the outer walls of the exodermal passage cells (Pridgeon *et al.* 1983). Tilosomes were also absent in *E. anceps*, *E. nocturnum* and *E. secundum* (Moreira *et al.* 2009; Oliveira & Sajo 1999). The cortex in *E. radicans* is parenchymatous, and the presence of pelotons

of the mycorrhizal fungi was restricted to the cells of cortex that were in contact with the substrate (Thangavelu & Ayyasamy 2017). Similar observations were made by Moreira & Isaias (2008) in *Sophronitis pumila* (Hook) Van den Berg & M.W. Chase, *Prescottia montana* Barb. Rodr., *Habenaria petalodes* Lindl., and *Polystachya estrellensis* Rchb. f.

The endodermis in *E. radicans* is uniseriate and its cells are thickly-walled. The thick-walled endodermal cells are interrupted by the presence of thin-walled passage cells. The endodermal thickenings form an efficient apoplastic barrier for the transfer of water and nutrients (Ma & Peterson 2003; Moriera & Isaias 2008). Hence the presence of passage cells is as essential in the endodermis as in the exodermis.

The vascular tissues in *E. radicans* are embedded in sclerenchymatous tissues, as it has been observed in many epiphytic orchids. This anatomical feature is considered to be a highly significant character that is related to the endurance of the plant during drought conditions (Nawaz *et al.* 2013; Thangavelu & Ayyasamy 2017). The pith is sclerenchymatous but the cells in the center are thinly-walled with small intercellular spaces.

Terrestrial orchids usually develop less anatomical and morphological adaptations for water stress. Nevertheless, an analysis of the vegetative structures discussed above clearly shows that *E. radicans* possess several anatomical adaptations that could aid in the survival of this orchid in stressful habitats. The presence of thick cuticle on adaxial and abaxial surfaces of the leaf and the presence of adaxial stomata substantiate the xeromorphic conditions tolerated by the plant. Velamen tissue in roots is basically important for the absorption of water and nutrients. Velamen in *E. radicans* resembles those of epiphytic than terrestrial orchids. Similarly, the nature of exodermis and endodermis is significant to provide mechanical protection and prevents evaporation of water. The absence of special storage organs in *E. radicans* is compensated by presence of the storage idioblasts in almost all the vegetative parts of this orchid. However, further experimental studies examining the

growth of this orchid in different environment and on different substrates would reveal the morphological plasticity of this orchid.

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