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Exodermis structure controls fungal invasion in the leafless epiphytic orchid *Dendrophylax lindenii* (Lindl.) Benth. *ex* Rolfe

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ABSTRACT

Leafless and shootless epiphytic orchids rely essentially on CAM photosynthesis in roots for carbon gain. However, it is believed that a proportion of carbon is obtained by endomycorrhizal associations. In this study, we show that *Dendrophylax lindenii* possesses a dimorphic exodermis with smaller, thin-walled passage cells that are depleted in flavonoids. No hyphae succeeded in penetrating into the cortex from a non-passage cell, but 20% of the hyphae in contact with a passage cell managed to penetrate into the cortex. The passage cells represent 40% of the amount of cells in the centre of the side that touches the substrate, but no passage cells are observed in the upper side of the root. This distribution and density of exodermal passage cells define a strategy for controlling the extent and location of fungal invasion in the orchid root. This strategy provides a mechanism for restricting fungal growth to the lower cortex and thus maximising carbon gain from photosynthesis while enabling further trophic exchanges from mycorrhizal associations.

Introduction

Mycorrhiza are a key element in plant–fungi symbioses that arose in the late Silurian/early Devonian and are thought to have been of paramount importance in the conquest of the land by plants (Remy et al., 1994; Stubblefield and Taylor, 1988; Taylor et al., 1995). Over 80% of all angiosperm species possess mycorrhizal associations (Smith and Read, 1997). In Orchidaceae Jussieu, the most diverse angiosperm family with distinctly more than 26,000 species described (The Plant List, 2010), mycorrhizal associations are present in most species (Arditti, 1992). One of the peculiarities of orchids with regards to mycorrhizae lies in their germination physiology: orchid seeds are very small and possess little nutritive reserve (Arditti, 1967, 1992; Arditti et al., 1990; Leake, 1994). As a result, all species are dependent upon mycorrhizal association for germination and establishment of protocorms, a structure that develops following germination and is myco-heterotrophic until the first leaves develop (Arditti, 1967, 1992). Following the establishment of the plantlet, mycorrhizal associations generally persist throughout the life of the orchid, with the rare exceptions of some tropical epiphytic species (Smith and Read, 2008). The distinctiveness of orchid mycorrhiza was early noticed when Bernard (1908) identified saprophytic or pathogenic *Rhizoctonia*-like fungi forming endomycorrhizae with cultures of protocorms. In contrast to the majority of vascular plants, which form vesicular-arbuscular mycorrhiza (VAM) with Glomeromycota, the fungal partner has shifted in the common ancestor of Orchidaceae from Glomeromycetes fungi to a particular clade of Basidiomycota, the order Cantharellales (Yukawa et al., 2009). Most orchid mycorrhizae occur with Cantharellales fungi and are referred to as Orchid Mycorrhizae (OM): Yukawa et al. (2009); Rasmussen (2002). Furthermore, accumulating evidence suggests that the relationship between orchids and fungi is not bi-directional and that orchids do not pay back fungi in carbon (Rasmussen and Rasmussen, 2009). However, recent evidence indicates that bi-directionality (i.e. mutualism) occurs at least in some ectomycorrhizal species (Cameron et al., 2008). Unique associations with these free-living saprophytic, often pathogenic Basidiomycotean fungi are thought to have broadened the ecological niche of orchids and may have had an important role in the evolutionary success of Orchidaceae (Ogura-Tsujita et al., 2009). Not surprisingly, 69% of all Orchidaceae are epiphytic species (Zotz, 2013), the remainder being terrestrial and a smaller fraction lithophytes (Dearnaley, 2007). Most orchids are leafy and only partially myco-heterotrophic, however over 100 species of orchids are fully achlorophyllous (Leake, 2005) and are henceforth myco-heterotrophic. A few terrestrial, often but not always achlorophyllous orchids, forms ectomycorrhiza (Girlanda et al., 2006; Liebel et al., 2010; Zimmer et al., 2007) but it is clear that this has been secondarily acquired during orchid evolution since

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in all cases they share an OM-forming common ancestor (Yukawa et al., 2009). This idea has also been conveyed by the identification of partially myco-heterotrophic orchids that are associated with trees through ectomycorrhiza but can simultaneously associate with *Rhizoctonia*-forming fungi (Bidartondo et al., 2004).

Although the mycorrhizal status of several terrestrial leafless orchids (e.g. *Corallorhiza trifida*) has been investigated, little is known about the mycorrhizae of leafless epiphytic orchids. Epiphytic leafless orchids occur in three subtribes of the tribe Vandae (subfamily Epidendroideae), in total 283 species: Aeridinae (*Taeniophyllum*, *Chiloschista*, *Microtatorchis* and *Phalaenopsis* section Aphyllae; in total 230 spp.), occurring mostly in Asia; Aerangidinae (*Microcoelia*, *Solenangis*, *Chauliodon*, in total 28 spp.) from Africa and Madagascar; and lastly Angraecinae with the New World genera *Campylocentrum* and *Dendrophylax* (Carlsward et al., 2006; Stewart et al., 2006; Summerhayes, 1943).

In this study, the anatomy and histochemistry of *Dendrophylax lindenii* (Lindl.) Benth. *ex* Rolfe roots has been examined. Comparison of the exodermis histology with the invasion pattern of fungal hyphae in the roots revealed a major role of the exodermis structure and histochemistry in the control of fungal invasion in the leafless epiphytic orchid *Dendrophylax lindenii*.

Materials and methods

Histochemistry and DPBA staining of D. lindenii roots

Dendrophylax lindenii (Fig. 1) root samples were collected in East Cuba, South of Guisa, Provincia of Granma, and stored in 70% ethanol. Hand sections of roots of *D. lindenii* were observed under a Nikon epifluorescence microscope eclipse E600 connected to a Nikon camera Ds-Fi 1. For histolocalisation of flavonoids, a B2A filter (excitation spectrum: 450–490 nm) was used in combination with diphenylboric acid 2-aminoethyl ester (DBPA) staining, which was performed as previously described (Sheahan and Rechnitz, 1992). For histolocalisation of lignin, a DAPI filter was used (excitation spectrum: 385–400 nm).

Image and statistical analysis

All measurements were effectuated using MacBiophotonics ImageJ (http://rsb.info.nih.gov/ij). For the quantification of flavonoid fluorescence in Fig. 4E, the outline of the outer cell wall of the exodermis portion shown has been traced with the segmented line tool of ImageJ. The plot profile function was used to obtain the fluorescence intensity values, expressed as grey value. The 3019 intensity values representing the profile were then retrieved of ImageJ and plotted against the corresponding distance (position) in Microsoft Excel. Significant statistical support for differences between two series was sought with Two-sample Student's *t*-tests. Two-sample Student's *t*-tests were performed in R (The R foundation for statistical computing). The null and alternative hypotheses were as follow (H_0): there is no difference in means between the two groups; (H_1): there is a difference in means between the two groups.

Results

Anatomy and histochemistry of Dendrophylax lindenii roots

Dendrophylax lindenii roots (Fig. 1) exhibit a clear polarity and bilateral symmetry with differentiated upper and lower sides. The velamen is two-layered, and the second layer is shed at the upper root side. Some cells of the epivelamen, that are in contact to the

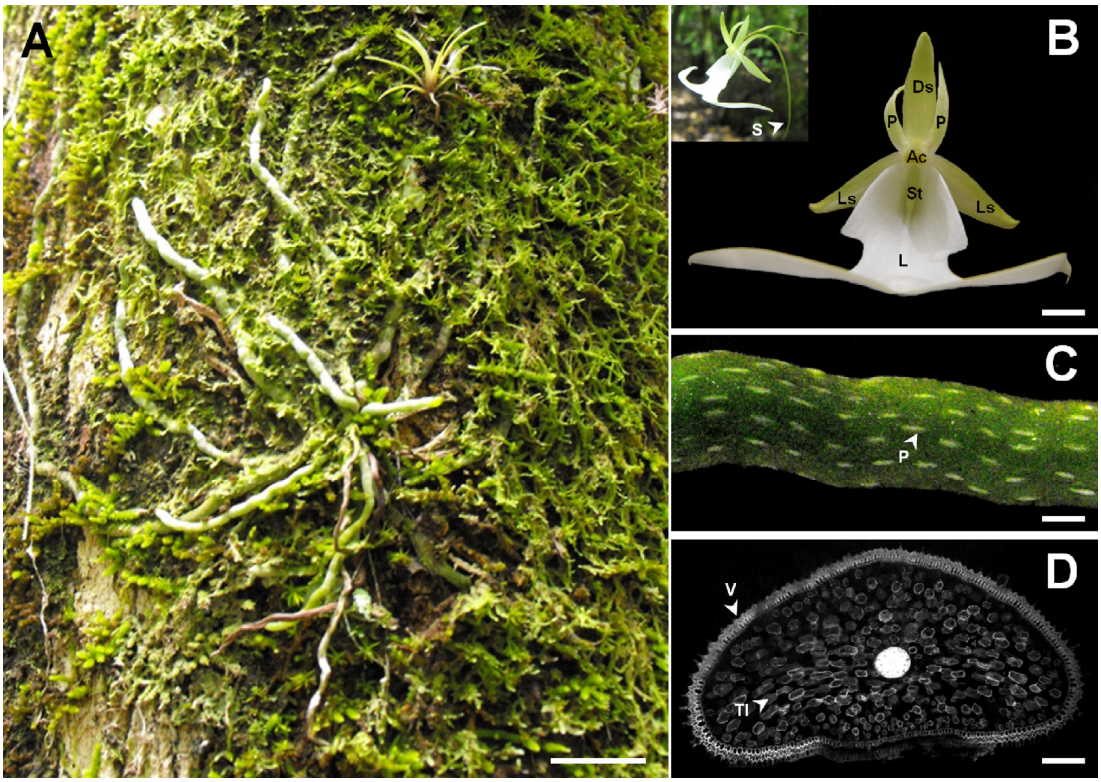

Fig. 1. Habit and morphology of *Dendrophylax lindenii*. (A) Habit and general morphology. (B) Flower morphology. Ds, distal sepal; Ls, lateral sepal; P, petal; L, labellum; Ac, anther cap; St, stigma; S, spur. (C) Root morphology. P, pneumatode. (D) Root cross-section. V, velamen; TI, tracheoidal idioblast. Scale bars: (A) 3 cm; (B) 0.5 cm; (C) 2.4 mm; (D) 0.7 mm.

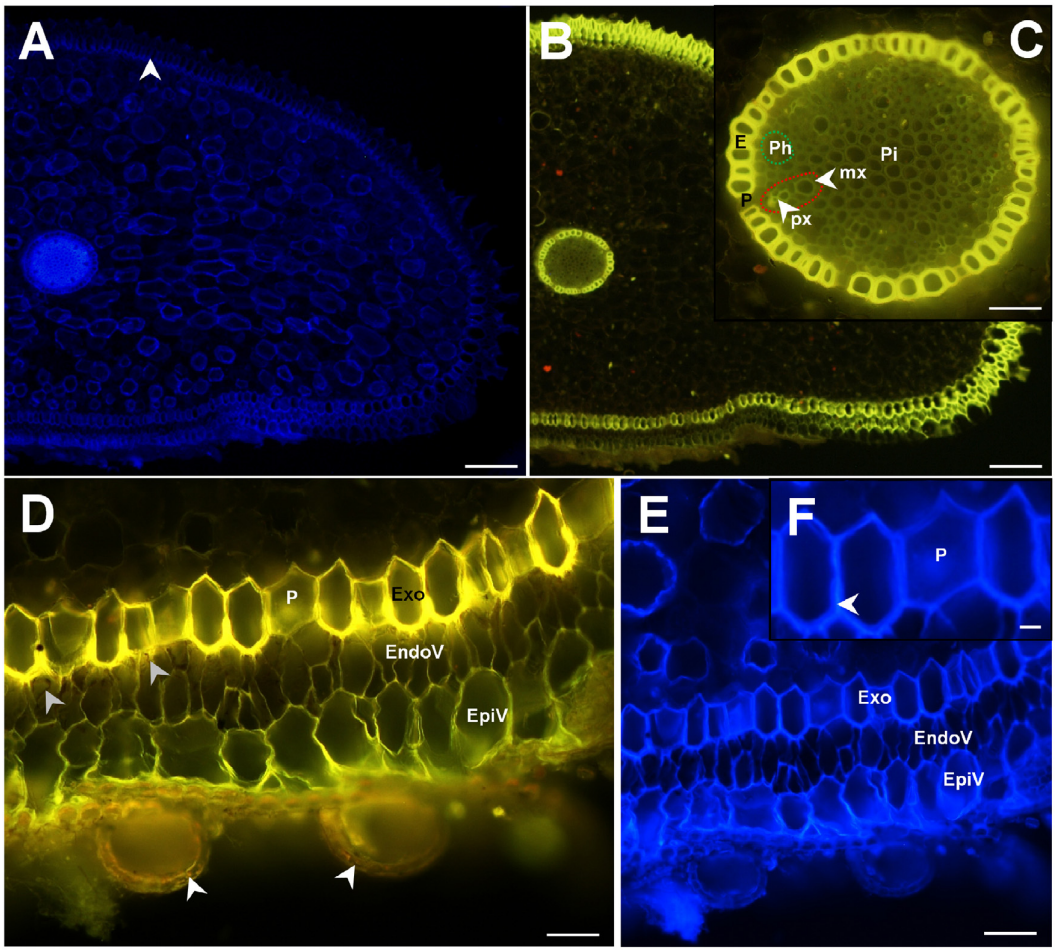

Fig. 2. Lignin and flavonoid distribution in *D. lindenii* root as revealed by histochemistry. (A) Root transection observed with DAPI filter, showing lignified cells. (B) Root transection observed with B2A filter and DPBA staining, revealing flavonoid distribution. (C) Flavonoids in the stele. E, endodermis; P, passage cell; Ph, phloem; px, protoxylem; mx, metaxylem; Pi, pith. (D) Flavonoid distribution in the lower root region. White arrowhead points to fungal sporangia and grey arrowhead points to intramatricial hyphae. Exo, exodermis; P, passage cell; EpiV, epivelamen; EndoV, endovelamen. (E) Lignin in the lower root region. (F) Close up of the exodermis. Arrowhead points to the secondary cell walls. Scale bars: (A and B) 350 μm; (C) 90 μm; (D) 65 μm; (E) 80 μm; (F) 12 μm.

support (=second (outer) velamen layer, V2), elongate and form root hairs which have the function of crampons. The endovelamen cell wall shows thick secondary cell walls thickening in the upper side of the root but is thin-walled in the lower side of the root. Endodermal cells are *O*-shaped.

Lignified tissues were visualised by histochemistry using a DAPI filter. In the stele, the xylem poles (7) exhibited the strongest signal. The endodermis was slightly lignified but essentially in the secondary cell walls (Fig. 2A). The whole cortex is photosynthetic, with a higher chloroplast concentration in the upper side of the root. Non-living tracheoidal idioblasts are interspersed into parenchymatous cells of the cortex and are lignified. The exodermis presents a distinct pattern in the upper and lower sides of the root, with lignin present in the lower parts of the secondary cell wall layers in the upper side (Fig. 2A, arrowhead) and in all cell walls in the lower side [Fig. 2A, E and F (arrowhead)]. In the passage cells of both endodermis and exodermis lignin is absent or very scarce (Fig. 2A, E and F). The first layer of the velamen or epivelamen is not lignified, but the second velamen layer – the endovelamen – is lignified in the central part of the lower side only (Fig. 2A and E).

The flavonoid distribution in *D. lindenii* root was examined using DPBA staining, which specifically binds to flavonoids (Sheahan and Rechnitz, 1992). The thick tertiary cell walls of the endodermis exhibit a strong yellow-gold fluorescence which is characteristic of flavonoids related to quercetin (Sheahan and Rechnitz, 1992). Flavonoids are essentially absent in the cortical cells, including tracheoidal idioblasts, but they are present in the thick tertiary cell walls of the outer part of the exodermis (Fig. 2B and D). The epivelamen is exempt of flavonoids in the central part of the lower side, but the flavonoid level subsequently increases dramatically towards the upper side and is correlated to a cell size increase (Fig. 2B). However, the fluorescence in the epivelamen is green-yellow, not yellow-gold as in the second velamen layer (Figs. 2B and 4A). This is a colour which is generally associated to kaempherol derivatives (Sheahan and Rechnitz, 1992). In the endovelamen, flavonoids are present in the central part of the lower side only and form a gradient which reaches a maximum at the point of contact between the orchid root and the support (Fig. 2B and D).

An OM-type fungus is associated with D. lindenii roots

Sporangial structures have been observed adjacent to the lower side of the root and numerous hyphae lie within the upper velamen and in the cortex as well (Figs. 2D and 3C–H). Arbuscular-like structures were present in the velamen (Fig. 3E and H), vesicular structures at the apex of a hypha were also observed (Fig. 3D and

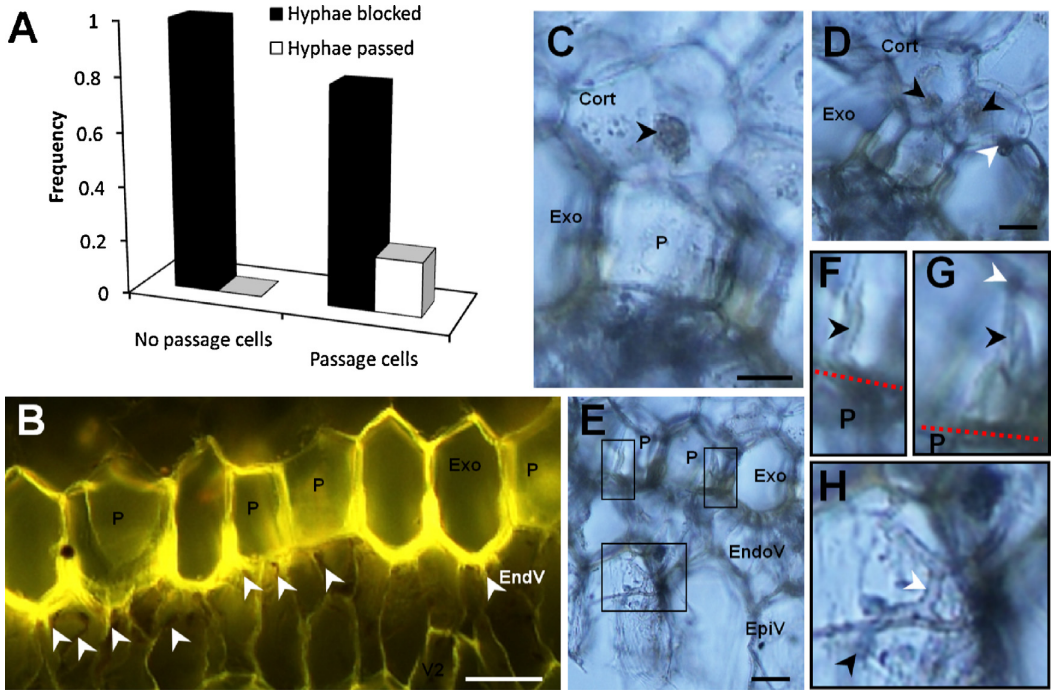

Fig. 3. Passage of hyphae through the exodermis layer and fungal structures. (A) Frequency of hyphae that fail or succeed to cross the exodermis. (B) Exodermal flavonoids and blocked hyphae endings (arrowheads). (C) Digested peloton in the cortex (arrowhead). (D) Pelotons in course of digestion in the cortex (black arrowheads). White arrowhead points a vesicular structure. (E) Hyphae that passed the exodermis. Exo, exodermis; P, passage cell; EpiV, epivelamen; EndoV, endovelamen. (F and G) Detail hyphae that crossed the exodermis. The red dotted line represents the exodermis outer wall. Black arrowhead point hyphae, white arrowhead points a vesicular structure that terminate a hypha. (H) Detail of mycorrhizal fungi in the velamen. White arrowhead points hypha, black arrowhead points an arbuscular structure. Abbreviations: Exo, Exodermis; Cort, Cortex; P, passage cell; EpiV, epivelamen; EndoV, endovelamen. Scale bars: (B and D) 25 μm; (C and E) 20 μm.

G). Importantly, digested pelotons or pelotons in course of digestion were present in cortical cells (Fig. 3C and D).

Most of the hyphae end upon touching the outer tangential cell wall of the exodermis (Figs. 2D and 3B). The hyphae 'blocked' at the exodermis versus those that succeeded to penetrate the exodermis barrier into the cortex were quantified (Fig. 3A). All hyphae ($n = 32$) that touched a non-passage cell (i.e. a standard exodermal cell) failed to penetrate further into the cortex (Fig. 3A). By contrast, when hyphae reached the exodermis by touching a passage cell, they penetrated the exodermis towards the cortex at a frequency of 0.2 ($n = 24$). Hence, penetration of the fungi into the cortex occurred only through a passage cell.

Passage cells occur primarily in the lower side of the exodermis in D. lindenii

In order to gain further insights into how *D. lindenii* controls fungal invasion, the distribution of passage cells was studied. By contrast to the endodermal passage cells which are regularly spaced and generally inserted very close to the protoxylem poles (Figs. 2C and 4B, D), the exodermal passage cells are irregularly repartitioned around the exodermis (Fig. 4A, C and D). Exodermal passage cells have a maximum density of 40% of the total amount of exodermal cells in the central zone of the lower side of the root (LOW1): Fig. 4D. The density of exodermal cells diminishes from the lower centre side of the root (LOW1) to the side of the root (region S2) where passage cells represent 4.5% of the exodermal cells (Fig. 4A, C and E). Exodermal passage cells were absent from the upper side regions (UP1 and UP2): Fig. 4A, C and E.

In order to test the hypothesis that cell wall flavonoids were involved in the exodermal-mediated restriction of fungal invasion, the flavonoid intensity profile of the exodermis outer surface was determined in a portion of the central lower zone (LOW1), where the density of passage cells is highest (Fig. 4E). The flavonoid fluorescence pattern depicted the exodermal cell types with saturating intensity for non-passage cells and low intensity for passage cells (Fig. 4E). Given that fungal hyphae were found to be able to penetrate into the cortex only through passage cells (Fig. 3A), the probability of fungal hyphae penetration was thus also correlated with flavonoid level.

Passage cells were significantly smaller than non-passage cells (Fig. 4G; *t*-test, $p < 0.0001$) and generally elongated by contrast to the more isodiametric shape of non-passage cells (Fig. 3B). A very few passage cells exhibited an isodiametric shape and were similar to the non-passage cells (e.g. Fig. 2F). They were only distinguished by thin cell walls and depletion in lignin and most notably flavonoids.

The exodermis cell width fluctuates but follows distinctive trends from the ab1 to the AD2 region (Fig. 4F and H). From ab1 to s1, exodermal cell length increases linearly (Fig. 4F and H), ab2 exodermal cells being significantly longer than ab1 cells (*t*-test, $p < 0.01$) and s1 cells being significantly longer than ab1 cells (*t*-test, $p < 0.0001$) as well as ab2 cells (*t*-test, $p < 0.01$).

Discussion

Anatomy of the Dendrophylax lindenii root

Our anatomical analyses confirmed the earlier account of Benzing et al. (1983) who first reported the 2-layered structure and shedding of the epivelamen on the upper side of the root in this species. The lignification of tracheoideal elements found in D. *lindenii* contrasts with the claims of Benzing et al. (1983) who stated that "[tracheoidal idioblasts] are not lignified or suberised,

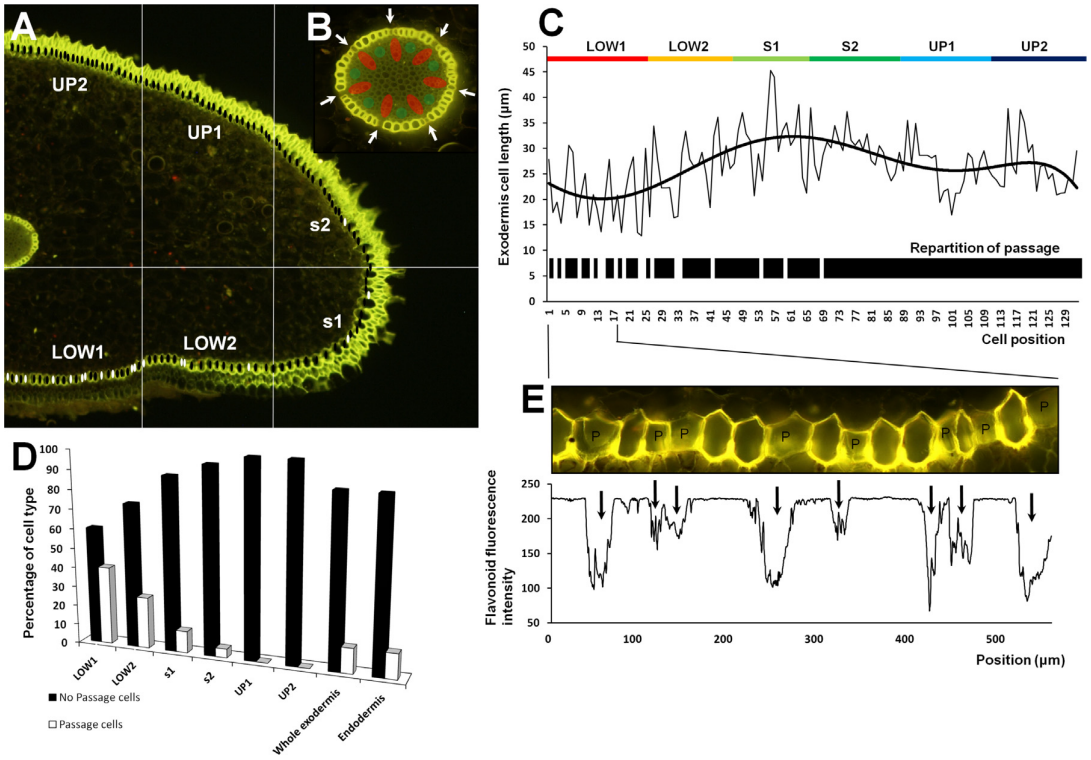

Fig. 4. Structure and flavonoid profile of the exodermis in *D. lindenii*. (A) Distribution of passage cells (white dot) in comparison to non-passage cells (black dots) in the exodermis. (B) Distribution of passage cells in the endodermis (arrowheads), the xylem poles are marked in red and the phloem in green. (C) Exodermis cell size and passage cell repartition. (D) Percentage of passage cells and no passage cells in the distinct domains of the exodermis and in the endodermis. (E) Flavonoid fluorescence intensity profile along the lower surface of the exodermis. The fluorescence intensity is represented as grey value. *Abbreviations*: LOW1, domain lower side 1; LOW2, domain lower side 2; S1, domain side 1; S2, domain side 2; UP1, domain upper side 1; UP2, domain upper side 2. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

but may develop irregular secondary thickenings resembling the wall sculpturing of some water-conducting cells". The lignifications of these tracheoideal elements likely play a role in water conduction within the wide photosynthetic parenchyma of the root. The size and shape difference in the exodermis is reminiscent of the dimorphic type of an exodermis that was defined by von Guttenberg (1968). The presence of certain passage cells with the morphology of non-passage cells indicates certain plasticity during exodermis development.

Dendrophylax lindenii forms mycorrhiza with a putative Basidiomycete fungus

The combination of mycorrhizal structures observed (pelotons, sporangia, intramatricial hyphae, arbusculoid hyphae, vesicular structures) indicates that the observed fungus forms mycorrhizal associations with *D. lindenii*. It is noteworthy that pelotons are a distinctive feature of orchid mycorrhiza, although they are not specific to them (Dearnaley, 2007). Furthermore, the digested states of pelotons denote a high activity of antifungal proteins such as chitinases. Interestingly, Bermudes and Benzing (1989) found pelotons and hyphal coils in cortical cells of two species of *Campylocentrum* (*C. fasciola* and *C. micrantha*), the sister genus to *Dendrophylax*. Likewise, Otero et al. (2002) showed that *Campylocentrum fasciola* and *C. filiforme* form mycorrhiza with bi-nucleate *Rhizoctonia*-like fungi of the genus *Ceratobasidium*. Endomycorrhiza in orchids (i.e. orchid mycorrhiza) is distinct in that it involves Basidiomycetes from the order Cantharellales, mostly from the families Ceratobasidiaceae, Tulasnellaceae and Sebacinaceae (Arditti, 1992; Yukawa et al., 2009). Furthermore, there is a phylogenetic signal for the occurrence of Ceratobasidiaceae as mycorrhizal partners in Angraecinae (Yukawa et al., 2009). The type and morphology of fungal structures observed in *Dendrophylax* and the phylogenetic signal in Angraecinae altogether suggest that *Dendrophylax lindenii* forms mycorrhiza with a Basidiomycete fungus of the Ceratobasidiaceae, although clearly this needs to be confirmed by a molecular study.

Exodermal structure controls fungal penetration: a role for flavonoids

Our study reinforces the idea that the exodermis plays a critical role in the control of fungal invasion. Non-passage cells of the exodermis are very proficient in preventing the penetration of fungal hyphae into the cortex (Fig. 3A). Passage cells have a checkpoint role in fungal invasion as they are the only possible points of entry for fungal hyphae. Likewise, only 20% of hyphae reaching a passage cell succeeded in passing through the exodermis (Fig. 3A). This echoes back to the results of Esnault et al. (1994), who found a percentage of infected exodermal passage cells in three epiphytic orchid species (*Epidendrum radicans*, *Dendrobium kingianum* and *Stanhopea tigrina*) that ranged between 7.2% and 16.2%. Interestingly, they did not find any significant differences in the percentage of infected exodermal passage cells in young versus old root sectioned, suggesting that mycorrhizal invasion occurs early in root development. Likewise, it has been found in several species that phenylpropanoids associated with mycorrhiza, both cytoplasmic and cell wall bound, are constitutively accumulated in roots (Codignola et al., 1989; Piché et al., 1981).

It is clear that the exodermis has a function in both limiting the level of endomycorrhiza and preventing the entry of pathogenic fungi. How passage cells control fungal hyphae entry is, however, not entirely clear. It is well known that fungal hyphae are restricted to the cortex (Arditti, 1992; Waterman and Bidartondo, 2008). Interestingly, it is hypothesised that the thick phenolics-rich cell walls of the endodermis act as a physical barrier that impedes hyphae entry into the stele (Beyrle et al., 1995; Peterson and Enstone, 1996; Waterman and Bidartondo, 2008). Likewise, the correlation between phenylpropanoids and root fungal colonisation in mycorrhizae of Pinaceae led Weiss et al. (1999) to propose a role for these compounds in the restriction of fungal growth.

Cell walls were much thinner in *D. lindenii* exodermis passage cells than in non-passage exodermal cells (0.78 ± 0.14 versus 2.87 ± 0.58 μm, SD; *t*-test, $p < 0.0001$). This suggests that the thick phenolics-rich outer cell walls of the exodermal non-passage cells form a physical barrier for fungus hyphae, similarly as endodermis cells. However, cell wall thickness alone cannot be accounted for the control of fungal infection, firstly because cellulose is degraded by endomycorrhizal fungi (Hadley, 1969) and secondly as these outer cell walls are not extremely lignified (Fig. 2A and E–F). Moreover, the very high flavonoid level in these outer cell walls, that has been revealed by DPBA staining (Figs. 2B, D and 4E), suggests a role in the limitation of fungal infection, given that many flavonoids exhibit antifungal activity (Alcerito et al., 2002; Cushnie and Lamb, 2005). In particular, the important difference in flavonoid intensity in the outer cell wall of passage versus non-passage cells (Fig. 4E) suggests that flavonoids restrict fungal growth in non-passage cells. Interestingly, Shimura et al. (2007) identified a flavonoid (chrysin) that acts as an antifungal compound that limits the fungal infection in the orchid *Cypripedium macranthos* var. *rebunense*. However, the authors did not study the localisation of chrysin. It has been recurrently shown that flavonoids can be associated with the cell wall (Ficcara et al., 2002; Freitas et al., 2003; Le Bourvellec et al., 2005; Markham et al., 2000; Fig. 2B and D). It is therefore possible that the flavonoid compounds observed in the exodermis of *D. lindenii* possess an antifungal activity that increases the fungal resistance conferred by the mechanical properties of the cell wall.

The data presented suggest that both cell wall thickness and phenolic content is responsible for the fungal growth restriction of the *D. lindenii* exodermis. Passage cells would permit a proportion of the hyphae to penetrate into the cortex due to their thin cell wall and low flavonoid content.

The pattern of passage cell repartition defines a strategy for controlling fungal invasion

Independently of the mechanistic details regarding how the exodermal non-passage cells impede fungal hyphae penetration, the density and repartition of passage cells in the exodermis define a strategy for controlling mycorrhizal invasion. The density of passage cells reaches its maximum (40%) in the central lower region of the exodermis (Fig. 4A and C–D). No passage cells occur in the upper side region (Fig. 4A and C–D). This distribution of passage cells allows a proficient control of the fungal invasion in *D. lindenii* roots. Fungi can only penetrate from the side in contact with the substrate (i.e. the lower side), which greatly minimises the chances of damaging the photosynthetic parenchyma. This asymmetric distribution of exodermal passage cells allows an efficient spatial control of the fungal invasion in *D. lindenii* roots. In addition to the spatial control of fungal invasion enabled by the distribution of passage cells, the density of passage cells adds another regulatory level by playing a 'sieve' role in filtering the number of fungal hyphae penetrating into the cortex. This is even more accentuated when one considers that the outer surface of a passage cell is much smaller in the dimorphic exodermis (5–8 times smaller in *Phalaenopsis*; Peterson and Enstone, 1996). In summary, the lower repartition and density of passage cells in the exodermis restrict the fungal invasion to the centre of the lower side of the root as well as the number of hyphae that penetrate into the cortex. This strategy for controlling fungal invasion is likely to be more critical in leafless epiphytic orchids, as root photosynthesis is thought to be responsible for the majority of the carbon intake (Benzing et al., 1983). Although no studies have investigated photosynthesis in *Dendrophylax*, Winter et al. (1985) showed that a leafless species of *Campylocentrum* (the sister genus of *Dendrophylax*), possesses a similar pattern of CO_2 exchange as compared to a CAM leaf, suggesting that the pneumatodes are proficient in playing the role of stomata on these highly specialised roots.

Exodermis cell size and passage cells: differentiation gradients

It is noteworthy that there is a negative correlation between passage cell density and exodermis cell size in the lower/side domain (Fig. 4C). Moreover, the extreme majority of passage cells are smaller than 20 μm, but such small cells represent less than 3% in the side and upper domain of the exodermis. Since passage cells differ from non-passage cells by an early arrest in development (Peterson and Enstone, 1996), reduced cell expansion may be a pre-requisite to passage cell formation or – expressed differently, differential expansion of certain zones of the exodermis might repress passage cell formation by promoting differentiation.

Furthermore, it is interesting that although the repartition of passage cells is drastically different in the exodermis and the endodermis (Fig. 4A–C), the total proportion of passage cells is equivalent in the exodermis and the endodermis (Fig. 4D). This could indicate that a common developmental constraint might be present during histogenesis of the exodermis and the endodermis, both of which originating from the ground meristem.

Conclusion

This study revealed that cell type organisation and histochemistry in the exodermis play a critical role in controlling fungal invasion into the root cortex by preventing invasion on the upper side of the root in the leafless and shootless epiphytic orchid *Dendrophylax lindenii*. The distribution of exodermal cells can be viewed as a strategy increasing carbon gain of the photosynthesising roots by which fungal hyphes only selectively can invade the roots. It would be interesting to determine whether such a strategy can be generalised for species sharing this peculiar habit or whether this is a more taxonomically restricted adaptation.

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