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СООБЩЕНИЯ

MYCORRHIZAL COLONIZATION OF ROOT CORTEX WATER STORAGE CELLS OF EPIPHYTIC ORCHIDS

© 2025 N. Bibikov[a, #], E. Voronina[a], A. Eskov[a], M. Ignatov[a, b]

[a]*Faculty of Biology, Lomonosov Moscow State University*
Leninskie Gory, 1, Moscow, 119234, Russia
[b]*Tsitsin Main Botanical Garden of the Russian Academy of Sciences*
Botanicheskaya Str., 4, Moscow, 127276, Russia
[#]*e-mail: bibik0808@mail.ru*

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In contrast to terrestrial species, epiphytic orchids possess water storage elements as an adaptation to dry habitats. Tracheoidal elements are present in roots and are involved in the interaction with mycorrhizal fungi, which colonize orchid roots obligately. Lignified water storage cells are located in the cortex and perform the functional role of water storage. Among the lignified exodermis cells, thin-walled passage cells are present. These elements are essential for the exchange of water between the root and the environment. This study supports existing data indicating that passage cells are the only exodermis cells through which fungal hyphae can pass. It also presents evidence of water storage cells being colonized by mycorrhizal fungi and shows that lignified elements of cortex are less conducive to peloton formation than thin-walled cortex cells.

Keywords: orchid mycorrhiza, water storage cells, root, epiphytic orchids

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Two major ecological groups of orchids, namely epiphytic and terrestrial, exhibit distinct ecological strategies and adaptations to the conditions they inhabit. Epiphytic orchids inhabit water-depleted conditions and possess certain anatomical elements that control water uptake. Epiphytic orchids possess a specific anatomical structure, the water storage cells (WSCs), which are found in the cortex of pseudobulbs, leaves, stems and roots. These lignified tracheoidal elements with secondary wall thickenings serve a function of water storage (Olatunji, Nengim, 1980; Balachandar et al., 2019, Li, Zhang, 2019; Ramesh et al., 2020). Another water-controlling structure located in the root is the exodermis, which is composed of cells with lignified walls. Among these cells, non-lignified passage cells (PCs) can be distinguished from exodermal cells by their size and shape (Porembski, Barthlott, 1988). Lignification is a defining feature of water-controlling elements in the cortex and exodermis, which can be clearly identified using fluorescence and confocal microscopy. A variety of commercially available stains are employed to differentiate between weakly and strongly lignified cells. Among these, alkaloid berberine has been demonstrated to yield optimal results (Brundrett et al., 1988; Joca et al., 2020).

The roots of epiphytic orchids are flattened from the abaxial side that provides plant's attachment to the substrate (Tay et al., 2021) and remains the point of fungal hyphae penetration into the root. All orchids are fully or partly dependent on fungal nutrition which is a supplementary or alternative to photosynthesis energy source for these plants (Cameron et al., 2008), and fungal communities associated with species of different strategies may differ drastically (Xing et al., 2019; Petrolli et al., 2021).

Fungal colonization of epiphytic orchid roots initiates by penetration of root hairs and velamen, which contains hyphae of both mycorrhizal and endophytic fungi. Exodermis appears to act as a control point for hyphal penetration. It has been

demonstrated that endophytes remain in the velamen, while mycorrhizal fungi pass through the exodermis (Pujasatria et al., 2022). Exodermal cells possess ∩-shaped cell wall thickenings and are unable to be colonized by mycorrhizal fungi. Exodermal cells are interspersed with thin-walled passage cells through which fungal hyphae reach the cortex (Porembski, Barthlott, 1988; Chomicki et al., 2014). PCs possess a function of water exchange between the root and the external environment, occur in exodermis and endodermis of various vascular plant taxa, including legumes and arbuscular mycorrhizal species and hence control hyphal growth in different types of plant-microbial endosymbiosis (Holbein et al., 2021). Nevertheless, PCs represent a vulnerability in plant mechanical defense, rendering plants susceptible to fungal pathogens that penetrate the exodermis in a manner analogous to mycorrhizal fungi (Koyyappurath et al., 2015).

Fungal colonization of root cortex is followed by formation of hyphal coils — pelotons inside cortex cells and massive colonization area occurs from the abaxial side. The cortex is composed of thin-walled cells and WSCs that possess secondary cell wall thickenings. Functional and structural heterogeneity of the epiphytic orchid root cortex prompts the question of whether the growth of fungal hyphae within the cortex is somehow controlled. The possibility of interaction between fungal hyphae and WSCs remains unclear. Presence of piths in WSCs secondary cell wall thickenings and ability of OM fungi to degrade lignin (Kohler et al., 2015; Miyauchi et al., 2020) suggest that WSCs may be colonized. The objective of this study was to investigate the rate of WSCs mycorrhizal colonization in order to shed light on the functions of these elements in mycorrhizal symbiosis.

MATERIALS AND METHODS

Samples of epiphytic orchids belonging to subfamily Epidendroideae were collected from Cát Tiên National Park, Vietnam in October 2015 and stored in 70% ethanol. Roots attached to phorophyte branches (substrate roots) were separated from plants and studied.

Hand-made sections of substrate roots were observed under Axioscop 40 FL fluorescence microscope with Axiocam (Carl Zeiss, Germany) using a 365 nm laser and Olympus FV-1000 laser confocal scanning microscope using a combination of 405 and 473 nm lasers with 0,01% berberine staining (berberine hemisulfate, Sigma-Aldrich) (Brundrett et al., 1988). A series of images was captured and subsequently merged into a single, three-dimensional image, or Z-stack, in order to obtain a comprehensive representation of the details within the image. The proportion of colonized WSCs and thin-walled cortex cells in the area of extensive fungal colonization was quantified.

RESULTS

Eight samples of epiphytic orchids were collected from phorophyte branches in Cát Tiên National Park. The samples included three plants belonging to the genera *Gastrochilus*, *Dendrobium*, and *Thrixspermum* and 5 plants belonging to unidentified genera of subfamily Epidendroideae.

Root and mycorrhiza anatomy of studied species

Substrate roots of all studied species exhibit a clearly distinguishable flattened abaxial side which is attached to the substrate (Fig. 1A). The velamen comprises two or more cell layers with root hairs. Exodermis consists of lignified cells elongated along longitudinal axis with ∩-shaped thickenings, among which non-lignified PCs of different size are present (Fig. 1B). The cortex is composed of two cell types: thin-walled cortex cells and lignified WSCs (Fig. 1A). Central part of the root is represented by the stele, which is separated from the cortex by the endodermis (Fig. 1A).

Fungal hyphae are present in root hairs and velamen on the abaxial side and exhibit irregular growth along the root axis (Fig. 1B, D). Cortex cells and WSCs contain functioning pelotons with hyphal structure or degraded pelotons lacking observable hyphae (Fig. 1A—C). Neither endodermis nor stele are colonized.

Structure and colonization of water storage cells

WSCs can be distinguished from non-lignified cortex cells on sections under berberine staining (Brundrett et al., 1988) and can be found in roots of all studied plants. The fluorescence of pelotons in WSCs is brighter than that of pelotons in thin-walled cells (Fig. 2).

At least six sections of each plant were examined for calculation. The proportion of colonized

thin-walled cortex cells and colonized WSCs was calculated in the area of massive colonization in order to demonstrate the difference in colonization rate between lignified and non-lignified cortex cells. For each sample either longitudinal or transverse sections were analyzed (Table 1, Fig. 3).

The mean share of colonized WSCs ranges from 1.6% (*Gastrochilus* sp.) to 11.9% (*Thrixspermum* sp.). Significant difference between shares of colonized WSCs and thin-walled cells (Student's t-test p-value < 0.01 for all samples) indicates that WSCs are colonized less frequently than cortex cells.

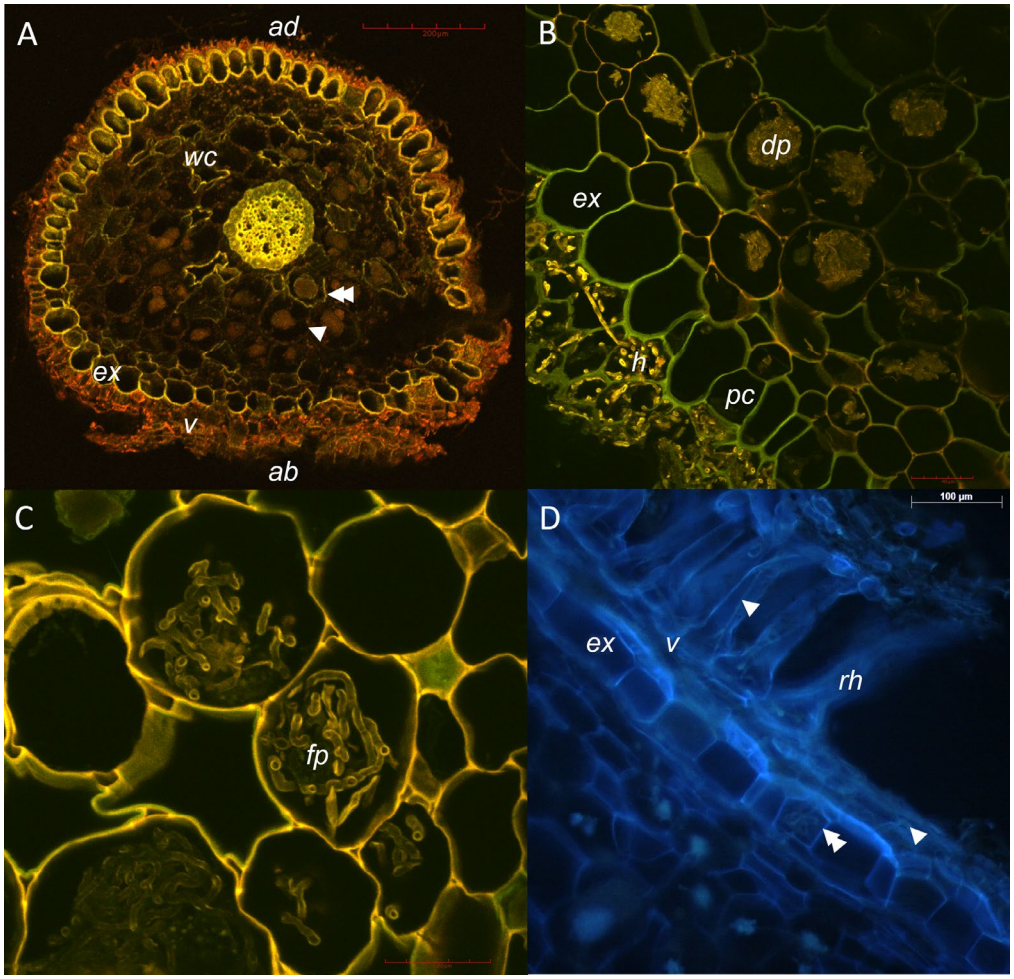

Fig. 1. Anatomy of fungal colonization in substrate roots of epiphytic orchids.

A — *Thrixspermum* sp. root on transverse section (*ab* — abaxial side, *ad* — adaxial side, *v* — velamen, *ex* — exodermis, *wc* — water storage cell, arrowheads point at colonized cortex cell, double arrowheads point at colonized WSC); B — root of Epidendroideae gen. sp. 4 on transverse section (*h* — fungal hyphae in velamen, *ex* — exodermis, *pc* — passage cell, *dp* — degraded peloton); C — root of Epidendroideae gen. sp. 4 on transverse section (*fp* — intact peloton); D — root of Epidendroideae gen. sp. 4 from abaxial side on longitudinal section (*rh* — root hair, *v* — velamen, *ex* — exodermis, arrowheads point at fungal hyphae in root hair and velamen, double arrowheads point at colonized PCs). Scale bars: A — 200 µm, B — 50 µm, C — 40 µm, D — 100 µm.

Рис. 1. Анатомия грибной колонизации субстратных корней эпифитных орхидных. А — поперечный срез корня *Thrixspermum* sp. (*ab* — абаксиальная сторона, *ad* — адаксиальная сторона, *v* — веламен, *ex* — экзодерма, *wc* — водозапасающая клетка, стрелки указывают на колонизированные клетки кортекса, двойные стрелки указывают на колонизированные водозапасающие клетки); В — поперечный срез корня Epidendroideae gen. sp. 4 (*h* — грибные гифы в веламене, *ex* — экзодерма, *pc* — пропускная клетка, *dp* — лизированный пелотон); С — поперечный срез корня Epidendroideae gen. sp. 4 (*fp* — интактный пелотон); D — продольный срез корня Epidendroideae gen. sp. 4, абаксиальная сторона (*rh* — корневой волосок, *v* — веламен, *ex* — экзодерма, стрелки указывают на грибные гифы в корневом волоске и веламене, двойные стрелки указывают на колонизированные пропускные клетки. Масштабные линейки: А — 200 мкм, В — 50 мкм, С — 40 мкм, D — 100 мкм.

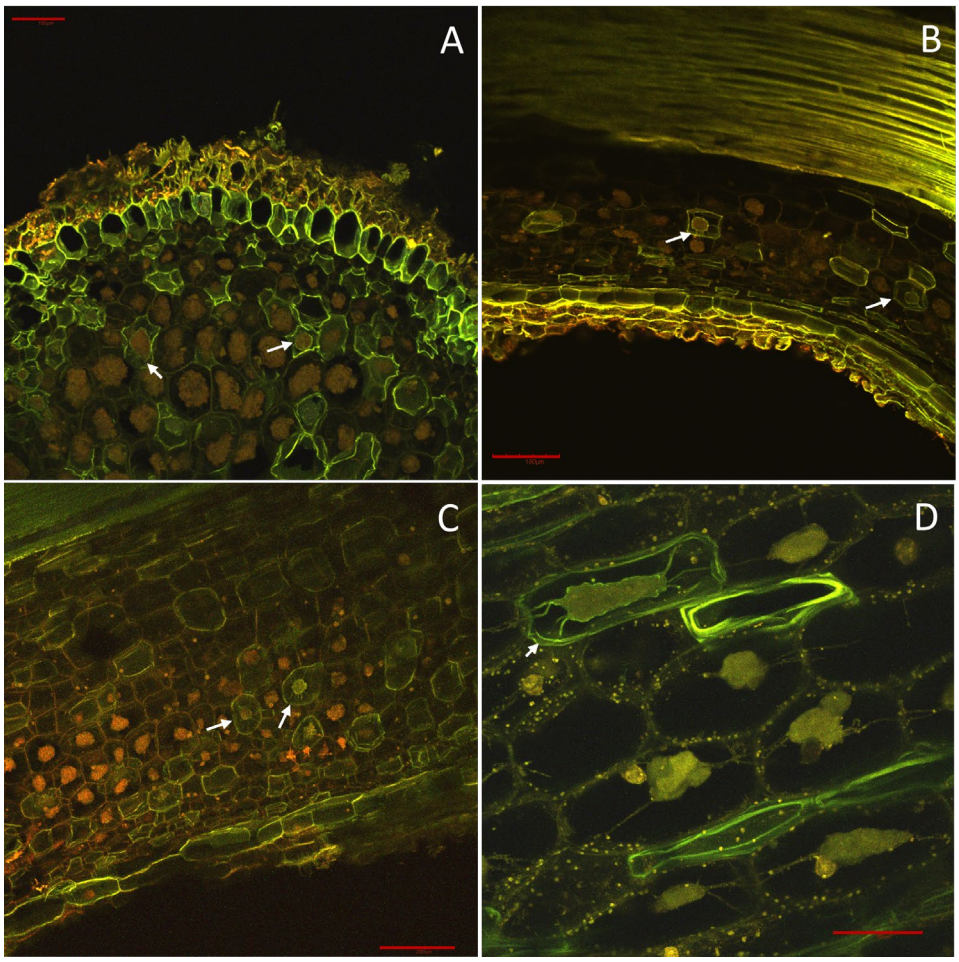

Fig. 2. Confocal images of colonized water storage cells in roots of studied plants.

A — Epidendroideae gen. sp. 3; B — Epidendroideae gen. sp. 4; C — Epidendroideae gen. sp. 5; D — *Thrixspermum* sp. Arrows point at WSCs with fungal pelotons. Scale bars: A — 100 μm; B — 180 μm, C — 200 μm, D — 60 μm.

Рис. 2. Конфокальные изображения колонизированных водозапасающих клеток в корнях изучаемых растений. A — Epidendroideae gen. sp. 3; B — Epidendroideae gen. sp. 4; C — Epidendroideae gen. sp. 5; D — *Thrixspermum* sp. Стрелки указывают на водозапасающие клетки с грибными пелотонами. Масштабные линейки: A — 100 μm; B — 180 μm, C — 200 μm, D — 60 μm.

Table 1. Analyzed sections and shares of colonized cortex cells

Таблица 1. Исследуемые срезы и доля колонизированных клеток кортекса

Name	Sample	Section	Colonized WSCs,%	Colonized thin-walled cells,%
Ep1	Epidendroideae 1	L	7.2 ± 0.75	60.2 ± 0.7
Ep2	Epidendroideae 2	T	7.8 ± 1.3	53.9 ± 5.9
Ep3	Epidendroideae 3	T	9.7 ± 1.4	55.5 ± 5.1
Ep4	Epidendroideae 4	L	10.9 ± 1.6	60.9 ± 6.0
Ep5	Epidendroideae 5	T	8.9 ± 0.7	59.3 ± 4.4
Dendr	*Dendrobium* sp.	T	6.9 ± 0.34	64.3 ± 0.9
Thrix	*Thrixspermum* sp.	T	11.9 ± 3.4	57.0 ± 3.8
Gastr	*Gastrochilus* sp.	T	1.6 ± 1.3	88.1 ± 2.9

Note. L — longitudinal, T — transverse.

Примечание. L — продольный, T — поперечный.

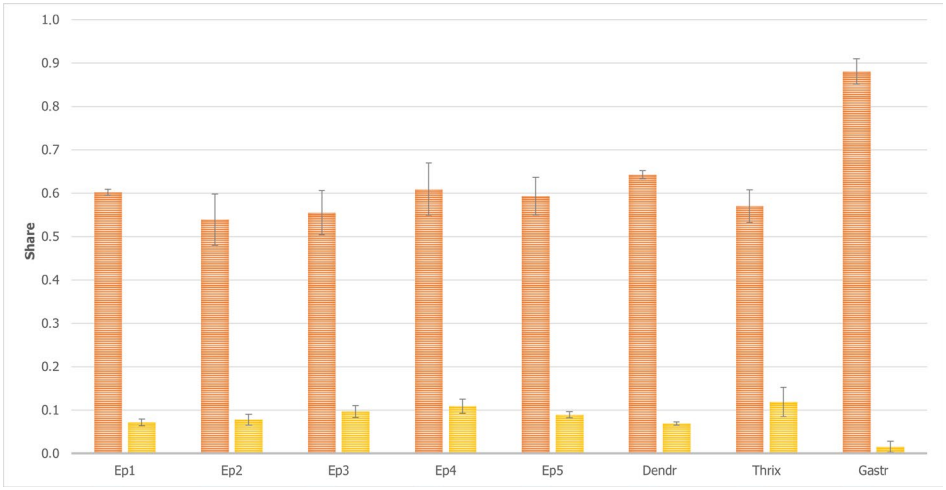

Fig. 3. Shares of colonized thin-walled cortex cells from total amount of thin-walled cortex cells (orange columns) and colonized WSCs from total amount of WSCs (yellow columns).

Рис. 3. Доля колонизированных тонкостенных клеток кортекса от общего числа тонкостенных клеток кортекса (оранжевые столбцы) и доля колонизированных водозапасающих клеток кортекса от общего числа водозапасающих клеток кортекса (желтые столбцы).

Structure and colonization of passage cells

Exodermal PCs are distinguished from exodermis cells by smaller size and the absence of ∩-shaped cell wall thickenings. In all studied samples, 100% of colonized exodermis cells were represented by PCs, therefore calculating the total PCs share was unnecessary to demonstrate a strong correlation between cell type and the presence of hyphae (Fig. 4).

DISCUSSION

In response to water depletion conditions, epiphytic orchid roots have evolved a unique loss prevention system comprising lignified exodermis and lignified WSCs in the cortex. The role of exodermal PCs in the control of fungal invasion is well documented for mycorrhizal and pathogenic fungi in a range of plant taxa (Porembski, Barthlott, 1988; Chomicki et al., 2014; Koyyappurath et al.,

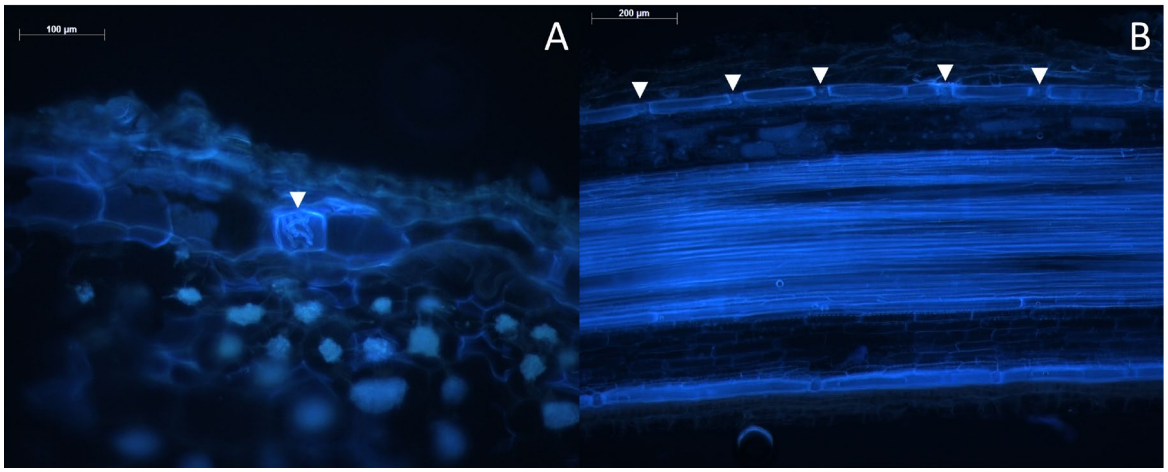

Fig. 4. Fluorescence images showing colonized PCs (arrowheads) on longitudinal sections.

Рис. 4. Флуоресцентные изображения колонизированных пропускных клеток (отмечены стрелками). A — Epidendroideae gen. sp. 4; B — *Dendrobium* sp.

2015). However, structural heterogeneity of root cortex has not yet been elucidated in the context of fungal infection control. In this study we present the first data on fungal colonization of these elements.

The capacity of fungi to colonize WSCs may be conditioned by either presence of piths in secondary wall thickenings, which facilitate the entry of hyphae into cells, or by their ability to degrade lignin. WSCs can be distinguished under confocal microscope by lignin autofluorescence enhanced with berberine. Furthermore, pelotons in WSCs exhibit brighter fluorescence than those in thin-walled cells. This may be attributed to local lignin degradation by fungi, although this hypothesis requires further investigation to be validated.

It seems probable that fungal colonization of WSCs is stochastic, given that the rate of colonization of WSCs is considerably lower than that of thin-walled cells. It is evident that lignified cell walls act as a barrier for hyphae, and therefore WSCs are less likely to be colonized than thin-walled cortex cells. The presence of additional factors that may deter fungi from colonizing WSCs remains to be investigated.

CONCLUSION

Studies on mycorrhiza anatomy shed light on mechanisms of plant-fungal interactions, elucidating the control of hyphal growth by plant. Although orchids do not possess specific cellular structures dedicated to fungal accommodation (not concerning the root itself), they do control hyphal growth within the root via mechanical barriers such as exodermis and endodermis. This study demonstrates that cortex WSCs are less conducive to fungal colonization than non-lignified cortex cells and support data that PCs are the only way to pass through exodermis. Further investigations in this area may be devoted to the possibility of WSCs lignin degradation by mycorrhizal or pathogenic fungi. Additionally, the presence of PCs in the endodermis of some orchid species suggests the possibility of stele colonization, for which the data is still very scarce.

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МИКОРИЗНАЯ КОЛОНИЗАЦИЯ ВОДОЗАПАСАЮЩИХ КЛЕТОК КОРЫ КОРНЕЙ ЭПИФИТНЫХ ОРХИДНЫХ

Н. М. Бибиков[1, *], Е. Ю. Воронина[1], А. К. Еськов[1], М. С. Игнатов[1, 2]

[1]Биологический факультет, Московский государственный университет им. М. В. Ломоносова
ул. Ленинские горы, 1, Москва, 119234, Россия
[2]Главный ботанический сад РАН им. Н. В. Цицина
ул. Ботаническая, 4, Москва, 127276, Россия
**e-mail: bibik0808@mail.ru*

В отличие от наземных орхидных, эпифитные виды обладают водозапасающими элементами, необходимыми для заселения сухих местообитаний. Трахеоидные элементы присутствуют в корнях и участвуют во взаимодействии с микоризными грибами, которые облигатно колонизируют корни орхидных. Лигнифицированные клетки расположены в коре и выполняют роль контроля водообмена. Среди лигнифицированных клеток экзодермы присутствуют тонкостенные пропускные клетки, необходимые для водообмена между корнем и окружающей средой. Данное исследование подтверждает существующие сведения о том, что пропускные клетки являются единственными клетками экзодермы, через которые могут проходить грибные гифы. Также представлены доказательства колонизации водозапасающих клеток микоризными грибами и показано, что лигнифицированные элементы коры менее благоприятны для формирования пелотонов, чем тонкостенные клетки коры.

Ключевые слова: микориза орхидных, водозапасающие клетки, корень, эпифитные орхидные