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Morpho-anatomical and molecular characterization of Isoberlinia doka ectomycorrhizal morphotypes in the Sudanian woodlands

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Abstract

Despite the ecological importance of *Isoberlinia doka* in the Sudanian woodlands of West Africa, its ectomycorrhizal (EcM) status and associated fungal community remain poorly documented. Here, we assessed the ectomycorrhizal status of *I. doka* and characterized its associated ectomycorrhizal morphotypes using an integrative morpho-anatomical and molecular approach. Fine roots were sampled from 12 forest reserves across the Sudanian and Sudano-Guinean climatic zones of Benin. Ectomycorrhizal root tips were identified microscopically, grouped into morphotypes based on standardized descriptive criteria, and taxonomically assigned using Sanger sequencing by targeting the internal transcribed spacer (ITS) region. Overall, 52.8% of the root samples were colonized by ectomycorrhizal fungi, confirming that *I. doka* consistently forms ectomycorrhizal associations in its natural range. However, colonization differed markedly between climatic zones, reaching 77.8% in the more humid Sudano-Guinean zone but only 27.8% in the drier Sudanian zone. Sixteen distinct ectomycorrhizal morphotypes were recognized, including *Russulales*, *Thelephorales*, Agaricales, *Sebacinales* and *Boletales*, with affinities to the genera *Lactifluus*, *Russula*, *Thelephora*, *Amanita*, *Scleroderma*, *Sebacina* and *Inocybe*. This study provides the first reference dataset for *I. doka* in Benin and offers a baseline for selecting locally adapted fungal inocula to support woodland restoration and regeneration under increasing seasonal drought.

1. Introduction

Ectomycorrhizal (EcM) symbioses are major determinants of nutrient uptake, water relations, and stress tolerance in many woody plants, and their characterization is therefore important for understanding forest functioning and supporting restoration practices (Brundrett 2009; Begum et al. 2019). Because many EcM fungi produce inconspicuous, hypogeous, or irregularly fruiting sporocarps, belowground approaches are often required to document the active fungal community. In this context, the combined use of morpho-anatomical characterization and molecular identification from ectomycorrhizal root tips remains one of the most reliable approaches for describing EcM diversity and establishing reference material for comparative studies (Agerer 1991; Horton and Bruns 2001).In tropical Africa, and particularly in West Africa, EcM fungal communities remain much less documented than in temperate ecosystems (Diédhiou et al. 2010; Bâ et al. 2011). Available studies have mainly focused on a limited number of host trees, including *Afzelia africana* and *Uapaca* spp., and standardized reference data for African EcM morphotypes are still scarce (Yorou et al. 2008a, b; Sanon et al. 2009). This knowledge gap limits both ecological interpretation of host–fungus associations and the identification of locally adapted fungal material for forest restoration.

Isoberlinia doka Craib & Stapf is a dominant tree species of West African Sudanian woodlands and an ecologically important component of these ecosystems. In Benin, the species is also of high socio-economic value but faces increasing anthropogenic pressure, while its belowground symbiotic associates remain poorly characterized. We hypothesized that Isoberlinia doka forms ectomycorrhizal associations with fungal partners that may have potential for use in restoration strategies. In this study,

we therefore (i) assessed the ectomycorrhizal status of *I. doka* in Benin and (ii) characterized its associated EcM morphotypes using an integrative morpho-anatomical and molecular approach. This work seeks to establish the first morpho-molecular reference framework for *I. doka*, thereby laying the foundation for ecologically informed restoration strategies in West African woodlands.

2. Materials and Methods

2.1 Study area and sampling design

The study was conducted in Benin (West Africa) in two contrasting climatic zones, the Sudanian (north of 10° N latitude) and the more humid Sudano-Guinean zones (between 7° and 10° N latitude), both characterized by *Isoberlinia doka*-dominated woodlands. Sampling was conducted in August 2024 and June 2025 in *I. doka*-dominated woodlands of the Sudanian and Sudano-Guinean zones of Benin. 06 forest reserves were selected per zone (12 in total). In each reserve, two sites at least 500 m apart were defined, and three mature *I. doka* trees (DBH > 10 cm) were randomly sampled per site, yielding 72 composite root samples. For each tree, fine roots bearing terminal tips were collected at four points around the base of the trunk and pooled into one composite sample. Root samples were cleaned, placed in sterile labeled containers, and transported to the laboratory within 24 h. Subsamples were preserved in FAA (Formalin Acetic acid-Alcohol 90:5:5 v/v/v) for morpho-anatomical analyses and in 2X CTAB buffer at − 20°C for DNA extraction.

2.2. Ectomycorrhizal status, colonization rate and morpho-anatomical characterization

Roots were washed with demineralised water, and terminal root tips were spread in Petri dishes containing 70% ethanol on a black background and examined under a stereomicroscope (Nikon SMZ800, 10–40×). Only healthy and intact terminal root tips were retained. Ectomycorrhizal (EcM) tips were identified according to standardized criteria, including colour change, branching pattern, presence of a continuous fungal mantle, and occurrence of emanating hyphae or rhizomorphs (Agerer 1991, 2001, 1997). When necessary, Congo Red and Calcofluor White were used to enhance external fungal structures (Ortiz-Ramírez et al. 2022). For each composite root sample, 20 terminal tips were randomly examined, giving a total of 1,440 root tips (72 samples × 20 tips). The four subsamples collected around each tree were pooled before observation. The mycorrhization rate (MR, %) was calculated as:

MR (%) = $\frac{number\ of\ sample\ mycorrhized}{total\ number\ of\ sample} X100$

Samples with confirmed EcM tips were retained for morpho-anatomical characterization. Root tips were grouped into morphotypes based on shared external morphology following standardized descriptive criteria (Agerer 1991; Rambold and Agerer 1997). Recorded characters included mantle colour, branching pattern, shape of unramified ends, presence and organization of rhizomorphs, mantle surface texture, and approximate size of the ectomycorrhizal system (Agerer 1991; Yorou et al. 2008a, b; Pritsch

et al. 2010). Representative root systems were first observed in 70% ethanol under the stereomicroscope; colours were determined using the Munsell colour chart, and dimensions were measured with Nikon NIS-Elements software. Additional fragments were mounted in 90% lactic acid to examine rhizomorphs and emanating hyphae. To observe mantle organization, root pieces were cleared in 14% sodium hypochlorite, and mantle fragments were peeled, mounted in 90% lactic acid, and occasionally stained with Congo Red (Agerer 1987–2006). Microscopic observations under transmitted light focused on mantle hyphal arrangement, hyphal diameter, septation, clamp connections, and other specialized cells when present (Rambold and Agerer 1997; Pritsch et al. 2010).

2.3 Root sectioning, staining and internal structure analysis

Representative root systems of each morphotype were used for anatomical characterization following standard ectomycorrhizal anatomical procedures (Agerer 1991; Jakucs et al. 2005). Two sectioning methods were applied: manual hand sectioning with a razor blade and vibratome sectioning (HM 650V) to obtain 30–50 µm transverse sections. Root segments were dehydrated through an ethanol series (70%, 85%, 100%), infiltrated with LR White resin, polymerized for 24 h at 60°C, and sectioned with a Leica UC6 ultramicrotome equipped with a diamond knife. Semi-thin sections were stained with 1% toluidine blue in 1% borax, rinsed, dried, and mounted in DePex. Congo red staining (1:50, 10 s) was also used to visualize and measure internal structures. Observations and image acquisition were performed with a Nikon DS-Fi3 camera mounted on a Nikon Ni-U compound microscope. These preparations were used to assess mantle organization and thickness, Hartig net penetration, and the general anatomical structure of ectomycorrhizal root tips (Agerer 1991; Pritsch et al. 2010). For each morphotype, three transverse sections were measured, giving a total of 48 sections. Recorded variables included mantle layering, mantle thickness (M), radial penetration depth of the Hartig net (H), and the number and size of epidermal and cortical cell layers. Total root cross-sectional area (S), mantle cross-sectional area (Sm), and the proportion of mantle area relative to total root area (Mp) were also calculated.

S = πR^2, where R is the root radius.

S_m = $\pi(R^2-(R-M)^2)$, where (M) is the mantle thickness

Mp= $\frac{R^2-(R-M)^2}{R^2}$ *x 100*

2.4 Molecular analyses, bioinformatics and statistical analysis

DNA was extracted from one to five root tips per morphotype using a CTAB-based protocol at CEMOFE, Ghent University, Belgium. To formally associate each, morphotype with a fungal taxon, special care was taken to ensure that each sample contained only a single taxon (a single morphotype). Host identity was verified on 12 samples (one per forest reserve) by amplification of the chloroplast rbcL gene (rbcLaF/rbcLaR) (Hasebe et al. 1994). Fungal symbionts were identified by amplification of the nuclear

ITS region using primers ITS1F/ITS4 (White et al. 1990). PCR products were checked on agarose gels, purified, and bidirectionally Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands).

Chromatograms were assembled and edited in Geneious Prime to obtain high-quality consensus sequences. ITS sequences were compared with GenBank and UNITE using BLASTn, and taxonomic assignments were accepted at species level for matches with ≥ 97% similarity and ≥ 90% query coverage; lower matches were interpreted conservatively at higher taxonomic levels. Differences in mycorrhization between the Sudanian and Sudano-Guinean zones were tested using a chi-square test. Quantitative morphometric and anatomical traits were summarized descriptively, while qualitative characters were assessed by descriptive comparison. Georeferenced data were processed in R using the **sf** package, and ectomycorrhizal occurrence hotspots were visualized by two-dimensional kernel density estimation and mapped with **ggplot2** 2 (Wickham, 2016). All analyses were performed in R 4.3.1.

3. Results

3.1 Ectomycorrhizal status of *Isoberlinia doka*

Host identity was confirmed for 12 representative root samples, corresponding to one sample per forest reserve. All of them were identified as *Isoberlinia doka*, with 99.8–100% identity to GenBank accessions (Table 1 in supplementary information). Microscopic examination confirmed that *I. doka* forms ectomycorrhizal associations in Benin. Ectomycorrhizal root tips were detected in 38 of the 72 root samples examined, indicating an overall colonization rate of 52.8% (Fig. 1A). Ectomycorrhizal occurrence differed significantly between climatic zones ($\chi^2 = 16.11$, $p = 0.00006$), with 77.8% of samples colonized in the more humid Sudano-Guinean zone compared with 27.8% in the drier Sudanian zone. Spatially, positive samples were concentrated in the central forest reserves, particularly Wari-Maro, Ouémé Supérieur, and Okpara, which therefore represent the main hotspots of ectomycorrhizal occurrence in the study area (Fig. 1B).

PD: Pendjari, GN: Goungoun, KT: Kota, LL: Lolo, ST: Sota, TR: Trois rivière, PS: Penessoulou, BS: Bassila, WM: Wari-Maro, OK: Okpara, KB: Kilibo, OS: Ouémé supérieur

3.2 Morphological and anatomical characterization of EcM fungal morphotypes

The 38 samples identified as mycorrhizal were classified into 16 distinct ectomycorrhizal morphotypes. Following the descriptive framework commonly used in detailed EcM fungal studies, these morphotypes can be interpreted at four complementary levels: external habit of the mycorrhizal system, nature of emanating elements, mantle organization, and internal root anatomy.

Mantle colour:

Mantle colour varied markedly among the *I. doka*'s EcM morphotypes, ranging from white to cream, yellow, orange, brown, dark brown, and nearly black (Fig. 2). Pale to whitish morphotypes were mainly represented by *Amanita* sp. (Fig. 2D), whereas yellow to orange tones were observed in *Scleroderma verrucosum*, *Amanita* sp., *Sebacina* sp., *Inocybe* sp., and *Lactifluus acrissimus* (Fig. 2A-C, K-L). Brown morphotypes were associated with *Russula congoana*, *Thelephora capitata*, and some unresolved taxa (Fig. 2H–J), while dark brown to black morphotypes characterized *Thelephora afrostuposa* and allied Thelephorales (Fig. 2E–G).

Ramifications and unramified ends:

Three main ramification types were recognized (Fig. 3: A-B-C): simple systems in *Amanita* sp. and *Russula congoana* (Fig. 3A), monopodial-pyramidal systems in *Thelephora afrostuposa* and *Inocybe* sp. (Fig. 3B), and monopodial-pinnate systems in *Sebacina* sp. and *Scleroderma* sp. (Fig. 3C). Unramified ends also varied among morphotypes, ranging from bent in *Thelephora capitata* and *Sebacina* sp. (Fig. 3D), to straight in *Lactifluus acrissimus* and *Lactifluus* sp. (Fig. 3E), and sinuous in *Inocybe* sp. (Fig. 3F).

Rhizomorphs, emanating hyphae, and mantle attachment:

Three rhizomorph shape were recognized: smooth-margined rhizomorphs in *Russula congoana* and *Amanita* sp. (Fig. 3F), hairy rhizomorphs in *Lactifluus acrissimus* (Fig. 3G), and interconnected filamentous rhizomorphs in Thelephorales and *Scleroderma verrucosum* (Fig. 3H). The mode of attachment to the mantle also differed among morphotypes, with hyphal fans in *Scleroderma verrucosum* (Fig. 3H), restricted-point connections in *Sebacina* sp. (Fig. 3I), and rhizomorphs growing off in flat angle in *Inocybe* sp. (Fig. 3J). Rhizomorph hyphal organization ranged from Undifferentiated with margins rather smooth and hyphae compacted in *Lactifluus acrissimus* (Fig. 3K), to Undifferentiated with hyphae loosely woven and of uniform diameter (*Thelephora afrostuposa*) (Fig. 3L), and rhizomorphs with clearly distinguishable individual hyphae in *Lactifluus luteopus* (Fig. 3M).

Structure of outer mantle layer (Fig. 3N-O-P-Q):

Observations distinguish both plectenchymatous and pseudoparenchymatous organizations within the present material. Irregular plectenchymatous mantles lacking a clearly defined hyphal pattern were observed in *Thelephora afrostuposa* and *Scleroderma* sp., where the hyphae are loosely arranged over the mantle surface, often with a preferential longitudinal orientation (Fig. 3N). In contrast, the *Russula* morphotype shows a denser plectenchymatous mantle composed of tightly interwoven, labyrinthiform hyphae (Fig. 3O). A distinct net-like plectenchymatous arrangement produced by multiply branched, squarrose hyphae characterize the *Amanita* morphotype (Fig. 3P). By contrast, the *Sebacina* morphotype exhibits a pseudoparenchymatous outer mantle composed of angular cells, in which individual hyphae are no longer clearly discernible (Fig. 3Q).

Internal anatomical characteristics:

Analysis of the cross-sections confirms the successful establishment of symbiosis in the examined root tips (Fig. 3: R-S-T). The anatomy reveals a well-developed fungal mantle surrounding the root apex and a distinct interface with the host's cortical tissues. The average thickness of the mantle is approximately 12 µm. The cross-sectional area of the fungal mantle (Sm) is approximately 0.07 mm², for a total root surface area of approximately 0.28 mm², representing a mantle proportion (Mp) of approximately 23.05%. Inside the root, an average of four layers of cortical cells are observed, between which the Hartig network penetrates to an average radial depth of 36.02 µm. In other words, the mantle accounted for nearly a quarter of the total cross-sectional area of the ectomycorrhiza, and the average depth of the Hartig network was approximately three times the average thickness of the mantle, indicating that the symbiotic interface extended well beyond the superficial fungal sheath into the root's absorptive tissues.

Mycorrhizal system length (Fig. 4, in supplementary information**)**:

The total length of the mycorrhizal system (a) ranged from 160 to 1108 µm, with both mean and median at 488 + 237 µm. The length of unramified ends (b) ranging from 21.8 to 417 µm, with a mean of 133 + 94 µm. The diameter of unramified ends (c) varied from 20.6 to 78.5 µm, with a mean of 44.2 + 16.2 µm, showing moderate variation but overall indicating rather robust terminal ends. The diameter of the axis (d) varied from 8.3 to 52.7 µm, with a mean of 24.3 µm.

Ramification types: A: simple, B: monopodial-pyramidal, C: monopodial-pinnate.

Unramified ends: D: bent, E: straight, F: sinuous. **Rhizomorph morphology**: G: smooth margins, H: hairy margins, I: interconnected filaments. Rhizomorph attachment to mantle: J: hyphal fans, K: restricted point, L: flat-angle emergence. **Rhizomorph internal organization**: M: compact undifferentiated hyphae, N: loosely woven hyphae of uniform diameter, O: distinct individual hyphae. **Outer mantle organization**: P: irregular plectenchymatous, Q: dense labyrinthiform plectenchymatous, R: net-like plectenchymatous, S: pseudoparenchymatous with angular cells. **Internal anatomy**: R: Root cross section view; S: Zoom on mantle and Hartig net; T: Longitudinal section of the mantle view. **Abbreviations**: (M) mantle; (H) Hartig net; (C) cortical cells; (X) xylem; (P) phloem; (m) mantle thickness; (h) Hartig net depth. **Scale bars**: A-E: 0.3 mm; F-J: 0.1 mm; K-M: 10 µm; N-Q: 10µm; R: 20µm; S: 10µm; T: 40µm.

3.3 Molecular identification of the EcM fungal morphotypes of *Isoberlinia doka*

Molecular analysis has provided taxonomic characterization for the 16 morphotypes grouped and described morphologically above (Table 2). At the community level, the fungal assemblage associated with *I. doka* encompasses the major EcM fungal lineages expected in tropical broadleaf systems, namely the *Russulales*, *Thelephorales*, *Agaricales*, *Sebacinales*, and *Boletales*. The results reveal a predominance of well-known tropical ectomycorrhizal genera. The morphotypes M10, M12, and M13 were assigned to *Lactifluus*, with a strong affinity for *L. acrissimus*; M16 showed an affinity for *Lactifluus luteopus*; and M3 was associated with *Russula congoana*, confirming the significant contribution of lineages related to the *Russulaceae* to the mycorrhizal spectrum of *I. doka*. Thelephorales were also well represented, notably by

EcM5W (*Thelephora capitata*) and M10 (*Thelephora afrostuposa*), while M9 and M1 were only partially resolved within *Thelephorales*-type affinities. Other genera of significant ecological and functional interest were confirmed, notably *Amanita* Sp. (M3, M4), *Scleroderma verrucosum* (M7, M14), *Sebacina* Sp. (M8), and *Inocybe* sp. (M15). The molecular dataset also exhibits a clear quantitative structure. Among the 16 morphotypes listed in Table 2, the *Russulaceae* sensu lato (*Russula* + *Lactifluus/Lactarius*) accounted for 37.5% of all morphotypes, the *Thelephorales* for 25%, *Amanita* 12.5%, *Scleroderma* 12.5%, and *Sebacina* and *Inocybe* 6.25%.

Table 2

Taxonomic assignment of the ectomycorrhizal morphotypes. M1 to M16 represent the 16 morphotypes describe

EcM fungal Morphotype	Voucher number	Best GenBank match (Taxon / Accession)	Identity (%)	Best UNITE match (Taxon / Accession)	Identity (%)
M1	TSC 25 – 13	*Uncultured Thelephorales*	94.81%	*Thelephorales* / DQ447990	94.81%
M2	TSC 24 – 20	*Russula congoana* / PQ552950	99.06%	*Russula congoana* / PQ552950	99.06%
M3	TSC 24– 112	*Amanita* sp / PQ552966	100%	*Amanita* sp / OP549270	97%
M4	TSC 24 – 11	*Thelephora capitata* / NR_119617	96.8%	*Thelephora capitata*/ DQ848612	96.77%
M5	TSC 24 – 3	*Amanita* sp / OM473966	97%	*Amanita* sp / OP549270	97%
M6	TSC 24– 118	*Uncultured Sebacina* sp/ KU141302	93.1%	*Sebacina* sp / KT461398	98.33%
M7	TSC 24– 113	*Scleroderma* sp / MZ027819	99.6%	*Scleroderma* sp / MZ027823	99.74%
M8	TSC 25– 45	*Thelephora afrostuposa* / NR_119954	95.2%	*Thelephora afrostuposa* / JF520431	95.23%
M9	TSC 24– 47	*Thelephora* sp / JX630827	97.61%	*Thelephoraceae* / JX630827	97.61%
M10	TSC 24– 49	*Lactifluus acrissimus* / KR364041	99.7%	*Lactifluus acrissimus* / KR364041	99.72%
M11	TSC 24– 189	*Lactarius tenellus* / KF133280	99.7%	*Lactarius tenellus* / LN849752	99.1%
M12	TSC 24– 178	*Lactifluus* sp / MZ027840	98.1%	*Lactifluus* sp / MZ027838	98.1%
M13	TSC 25– 162	*Lactifluus acrissimus* / PV135642	99.2%	*Lactifluus acrissimus* / KR364041	99.44%
M14	TSC 24– 114	*Scleroderma verrucosum* / FJ840458	99.84%	*Scleroderma* sp / MZ027824	99.33%
M15	TSC 25– 116	*Inocybe* sp / OQ448198	97.7%	*Inocybaceae.* / OQ448198	97.72%
M16	TSC 25– 111	*Lactifluus luteopus* / PV135606	90.5%	*Lactifluus luteopus* / KR364119	89.49%

4. Discussion

This study provides one of the first integrative morpho-anatomical and molecular characterizations of ectomycorrhizal (EcM) communities associated with *Isoberlinia doka* in Benin. The results show that *I. doka* supports a diverse EcM fungal community dominated by major African lineages, particularly Russulaceae and Thelephoraceae, together with relatively high colonization rates and substantial morphotype diversity. These findings confirm that *I. doka* maintains active EcM associations under Sudanian conditions and highlight the ecological importance of belowground fungal symbioses in West African woodlands. The relatively high colonization rate observed here is consistent with the functional role of EcM fungi in improving nutrient acquisition and water uptake in nutrient-poor tropical soils, thereby enhancing host performance under seasonal drought (Bâ et al. 2012). The strong contrast in colonization between the Sudano-Guinean and Sudanian zones further suggests that climatic conditions, and probably water availability, act as important environmental filters on EcM establishment and persistence. This interpretation agrees with previous studies from Africa showing that EcM fungal distribution is influenced by seasonality, host distribution, and local environmental conditions (Ducousso et al. 2003; Diédhiou et al. 2010; Bâ et al. 2011, 2012), as well as with broader evidence that tropical EcM communities are strongly structured by environmental filtering (Tedersoo et al. 2010, 2014; Corrales et al. 2018). In Benin, the higher occurrence of EcM fungi in the central Guineo-Sudanian transition zone is also consistent with the distribution of EcM host trees in this region (Yorou et al. 2001; Meidl et al. 2021), whereas drier northern areas appear more constrained by climatic stress and anthropogenic disturbance (Yorou et al. 2011; Teteli et al. 2024; Boukary et al. 2024).

The taxonomic composition recovered from *I. doka* is coherent with current knowledge of African EcM biogeography. The predominance of Russulaceae and Thelephoraceae agrees with previous fruit-body-based and molecular studies from African woodlands and forests, which have repeatedly identified *Russula*, *Lactifluus*, and thelephoroid fungi as major components of tropical African EcM communities (Verbeken and Buyck 2002; Diédhiou et al. 2010; Meidl et al. 2021; Tchan et al. 2023; Tondeleir et al. 2025; Kupagme et al. 2026). The morphotype diversity observed here is also comparable to that reported for other dominant African EcM hosts such as *Afzelia africana* and *Uapaca* spp. (Sanon et al. 2009; Bâ et al. 2012), reinforcing the view that Sudanian woodlands harbour rich but still insufficiently documented belowground fungal communities.

An important contribution of this study is the use of an integrative approach combining morphotyping and molecular identification. As shown in both African and temperate systems, morphology alone is useful for delimiting operational morphotypes but often insufficient for reliable taxonomic identification, especially where sporocarps are seasonal and reference data remain scarce (Horton and Bruns 2001). The presence of unresolved morphotypes in the present study reflects not only the underrepresentation of African EcM fungi in sequence databases, but also the limited availability of comparative morpho-anatomical descriptions. Some of these unresolved morphotypes may therefore represent poorly documented or even undescribed taxa, suggesting that the diversity of African EcM fungi is still underestimated. Moreover, several diagnostic characters, including mantle organization, rhizomorphs,

emanating hyphae, and Hartig net structure, may vary with ecology and developmental stage, particularly under seasonal drought and nutrient-poor conditions (Agerer 1991; Jakucs et al. 2005; Corrales et al. 2018; Liu et al. 2020). This further supports the need to combine detailed anatomical observations with DNA-based identification in tropical EcM studies.

From an applied perspective, these findings provide a first reference framework for *I. doka* EcM morphotypes and support the use of locally adapted EcM inocula in restoration and regeneration programmes. Conserving mature *I. doka* stands may therefore help preserve not only the host tree, but also an important reservoir of belowground fungal diversity. However, some limitations should be acknowledged. Taxonomic identification relied on a single barcode marker (ITS), species-level resolution remained constrained by incomplete African reference databases, and soil physicochemical variables were not assessed directly. Future studies combining multilocus sequencing, metabarcoding, soil characterization, and seasonal monitoring will help refine our understanding of the diversity and ecological functioning of EcM fungi associated with *I. doka*.

5. Conclusion

This study provides the first morpho-anatomical and molecular characterization of ectomycorrhizal symbioses associated with *Isoberlinia doka* in Benin. The results confirm that *I. doka* is an ectomycorrhizal host associated with a diverse fungal community, mainly represented by Russulales and Thelephorales. The marked difference in mycorrhization rates between the Sudano-Guinean and Sudanian zones suggests that climate, particularly water availability, strongly influences EcM establishment. These findings establish a first reference framework for *I. doka* morphotypes and support the use of locally adapted ectomycorrhizal inocula in restoration and regeneration programs.

Declarations

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Conflict of interest. The authors have declared that no competing interests exist.

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Author contributions. Conceptualization: TSC, AV, NYS; Data curation: TSC, DV; Formal analysis: TSC, BAO; Funding acquisition TSC, AV, NYS; Methodology: TSC, AV, NYS, PA, OL, BAO, DV; Project administration: AV, NYS; Supervision: AV, NYS; Validation: AV, NYS; Writing - original draft: TSC, DV; Writing - review and editing: TSC, BAO, OL, PA, DV, AV, NYS.

Data availability

The ITS sequences generated from the ectomycorrhizal (EcM) fungal morphotypes have been deposited in GenBank under accession numbers PZ577609-PZ577624.

References

1. Agerer R (1987–2006) *Colour atlas of ectomycorrhizae*. Einhorn-Verlag, Schwäbisch Gmünd
2. Agerer R (1991) Characterization of ectomycorrhizae. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza. Methods in Microbiology, vol 23. Academic, London, pp 25–73
3. Agerer R (2001) Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114
4. Bâ AM, Duponnois R et al (2011) *Les champignons ectomycorhiziens des arbres forestiers en Afrique de l'Ouest: méthodes d'étude, diversité, écologie, utilisation en foresterie et comestibilité*. IRD Éditions
5. Bâ AM, Duponnois R, Moyersoen B, Diédhiou AG (2012) Ectomycorrhizal symbiosis of tropical African trees. Mycorrhiza 22:1–29
6. Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1068
7. Boukary A-A, Tchan IK, Houdanon RD, Olou AB, Badou SA, Yahouedehou G, Hegbe ADMT, Yorou NS (2024) Microclimate and the spatio-temporal dynamics of natural production of two ectomycorrhizal fungi from the Guineo-Sudanian ecozone in Benin. Glob Ecol Conserv 55:e03238
8. Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77
9. Corrales A, Henkel TW, Smith ME (2018) Ectomycorrhizal associations in the tropics: biogeography, diversity patterns and ecosystem roles. New Phytol 220:1076–1091
10. Diédhiou AG, Selosse M-A, Galiana A, Diabaté M, Dreyfus B, Bâ AM, Miana de Faria S, Béna G (2010) Multi-host ectomycorrhizal fungi are predominant in a Guinean tropical rainforest and shared between canopy trees and seedlings. Environ Microbiol 12:2219–2232
11. Ducousso M, Bâ AM, Thoen D (2003) Les champignons ectomycorhiziens des forêts naturelles et des plantations d'Afrique de l'Ouest: une source de champignons comestibles. Bois Trop 275:51–63
12. Hasebe M, Omori T, Nakazawa M, Sano T, Kato M, Iwatsuki K (1994) rbcL gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc Natl Acad Sci USA 91:5730–5734
13. Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1871

14. Jakucs E, Erős-Honti Z, Agerer R (2005) Morphological-anatomical characterization and molecular identification of *Tomentella stuposa* ectomycorrhizae and related anatomotypes. Mycorrhiza 15:247–258
15. Kupagme JY, Põlme S, Yorou NS, Gohar D, Mikryukov V et al (2026) Continental-scale diversity of African soil fungi. Fungal Divers 136:136005
16. Liu Y, Li X, Kou Y (2020) Ectomycorrhizal fungi and forest ecosystems: spatiotemporal distribution and ecological effects. Forests 11:453
17. Meidl P, Furneaux B, Tchan KI, Kluting K, Ryberg M, Guissou M-L, Soro B, Traoré A, Konomou G, Yorou NS, Rosling A (2021) Soil fungal communities of ectomycorrhizal dominated woodlands across West Africa. MycoKeys 81:45–68
18. Ortiz-Ramírez JA, Cuéllar-Cruz M, López-Romero E (2022) Cell compensatory responses of fungi to damage of the cell wall induced by Calcofluor White and Congo Red with emphasis on *Sporothrix schenckii* and *Sporothrix globosa*. Front Cell Infect Microbiol 12:976924
19. Pebesma E (2018) Simple features for R: standardized support for spatial vector data. R J 10:439–446
20. Pritsch K, Becerra A, Põlme S, Tedersoo L, Schloter M, Agerer R (2010) Description and identification of *Alnus acuminata* ectomycorrhizae from Argentinean alder stands. Mycologia 102:1263–1273
21. Rambold G, Agerer R (1997) DEEMY – the concept of a characterization and determination system for ectomycorrhizae. Mycorrhiza 7:113–116
22. Sanon KB, Bâ AM, Delaruelle C, Duponnois R, Martin F (2009) Morphological and molecular analyses in *Scleroderma* species associated with some Caesalpinioid legumes, Dipterocarpaceae and Phyllanthaceae trees in southern Burkina Faso. Mycorrhiza 19:571–584
23. Tchan KI, Olou BA, Houwannou GB, Meidl P, Hegbe ADMT, Guissou M-L, Yorou NS (2023) Highlighting the diversity of the rhizosphere mycobiome of five native West African trees. Int J Biodivers Conserv 15:30–47
24. Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263
25. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV et al (2014) Global diversity and geography of soil fungi. Science 346:1256688
26. Teteli SC, Badou AS, Dramani R, De Kesel A, Sambieni KR, Diansambu MI, Kouton BN, Yorou SN (2024) Spatial analysis vis a vis local perception of anthropogenic pressures on natural habitats of ectomycorrhizal fungi in Wari-Maro Forest Reserve in Benin. Asian J Mycol 7:51–67
27. Tondeleir L, De Crop E, Semenova T, Nuytinck J, Njouonkou A-L, Guelly AK, Dierickx G, Geml J, Verbeken A (2025) Back to the roots: uncovering ectomycorrhizal communities across three major African vegetation types. IMA Fungus 16:e147055
28. Verbeken A, Buyck B (2002) Diversity and ecology of tropical ectomycorrhizal fungi in Africa. In: Watling R, Frankland JC, Ainsworth AM, Isaac S, Robinson CH (eds) Tropical mycology, vol 1. Macromycetes. CABI Publishing, Wallingford, pp 11–24

29. White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322
30. Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York
31. Yorou NS, De Kesel A (2011) Champignons supérieurs / Larger fungi. In: Neuenschwander P, Sinsin B, Goergen G (eds) Protection de la Nature en Afrique de l'Ouest: Une Liste Rouge pour le Bénin / Nature conservation in West Africa: Red List for Benin. International Institute of Tropical Agriculture, Ibadan, pp 47–61
32. Yorou NS, De Kesel A, Sinsin B, Codjia JTC (2001) Diversité et productivité des champignons comestibles de la forêt classée de Wari-Maro (Bénin, Afrique de l'Ouest). Syst Geogr Plants 71:613–625
33. Yorou NS, Agerer R, Raidl S (2008a) *Afzeliaerhiza beninensis* + *Afzelia africana* Sm. Descr Ectomyc 11/12:1–8
34. Yorou NS, Agerer R, Raidl S (2008b) *Uapacaerhiza wariensis* + *Uapaca guineensis* Müll Arg. Descr Ectomyc 11/12:147–153

Figures

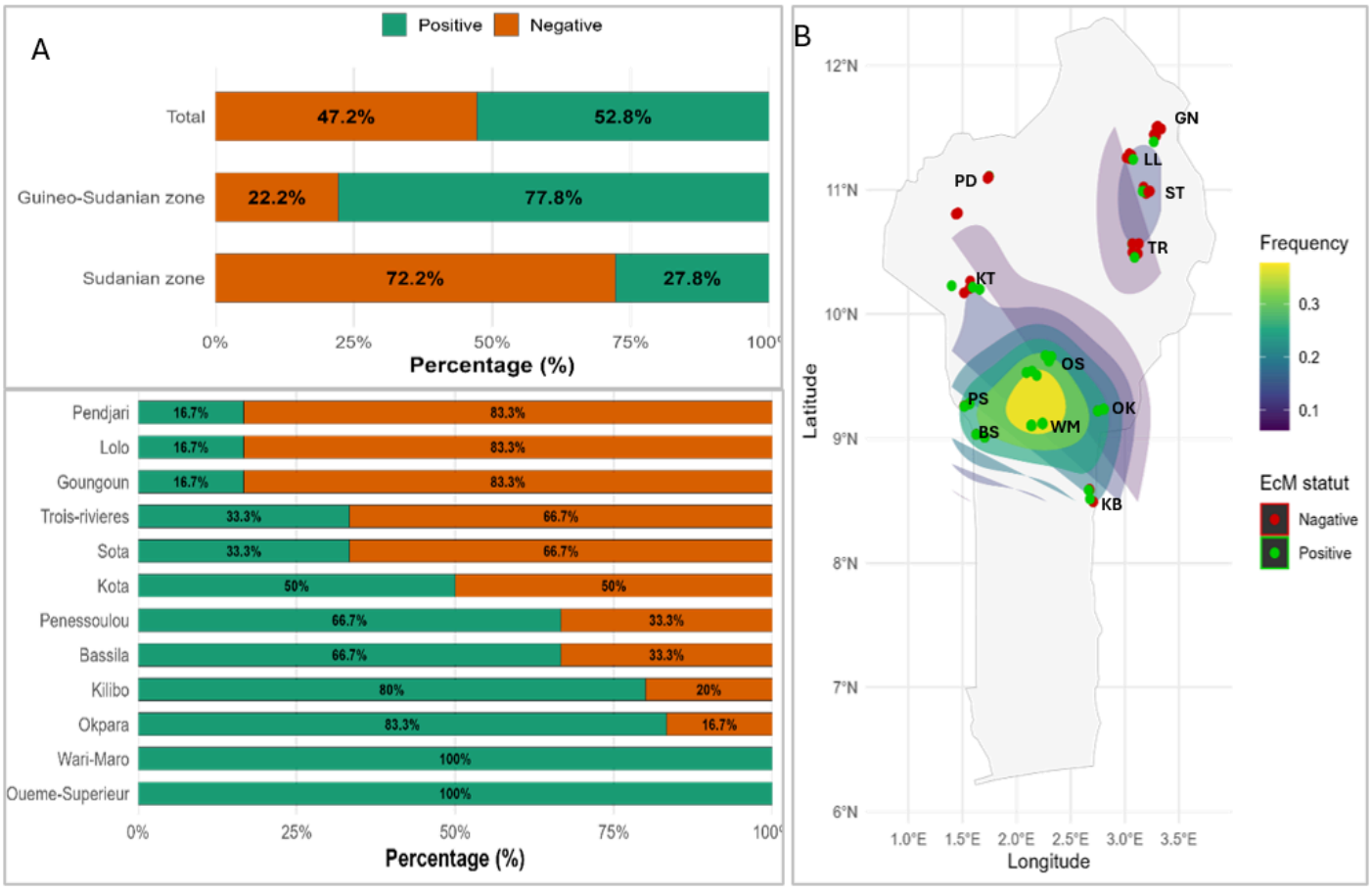


Figure 1

Occurrence of EcM fungal on *Isoberlinia doka* in Benin.

PD: Pendjari, GN: Goungoun, KT: Kota, LL: Lolo, ST: Sota, TR: Trois rivière, PS: Penessoulou, BS: Bassila, WM: Wari-Maro, OK: Okpara, KB: Kilibo, OS: Ouémé supérieur

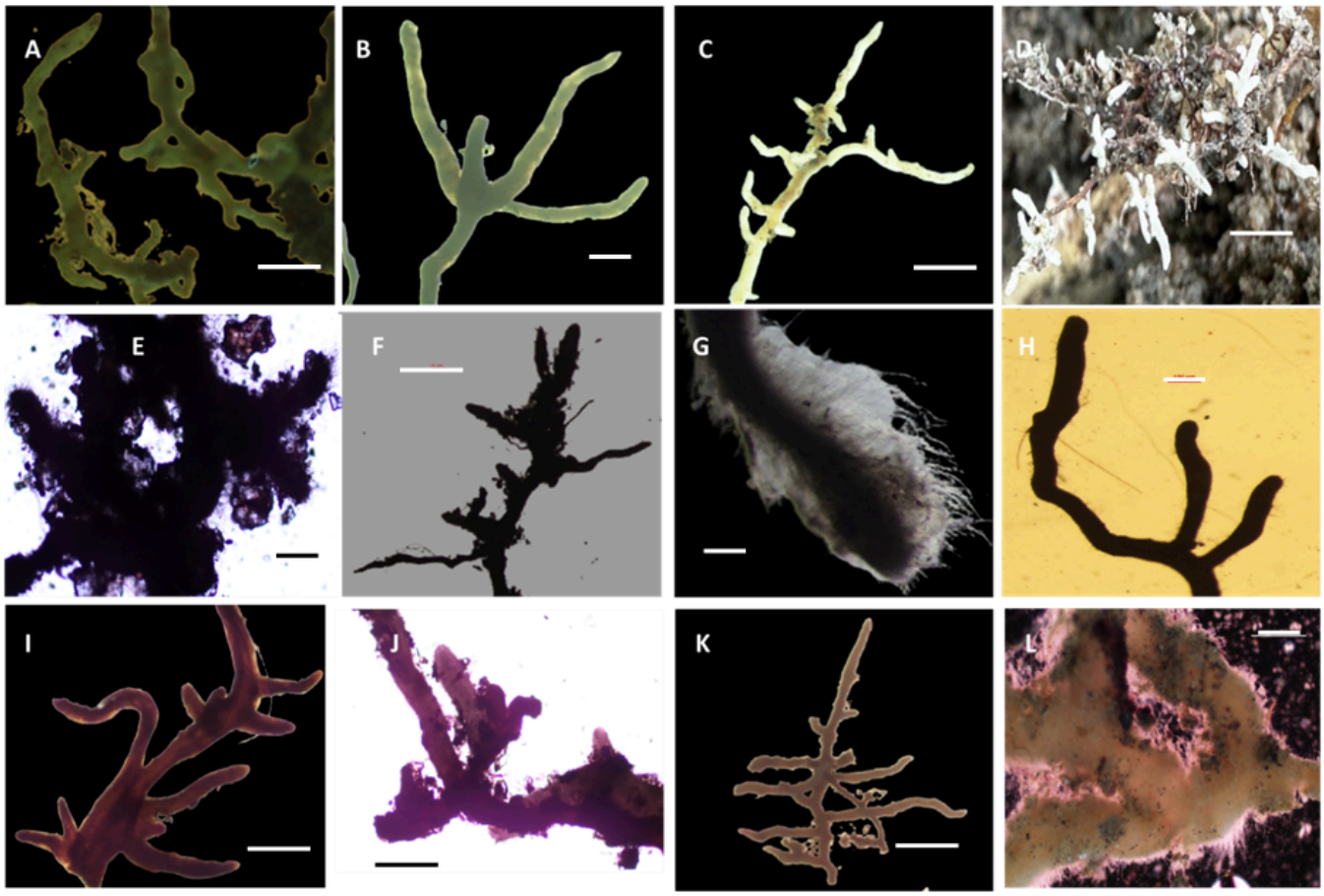


Figure 2

Representative mantle colour variation among ectomycorrhizal morphotypes associated with *Isoberlinia doka*. A: *Scleroderma verrucosum,* B: *Amanita* sp;C: *Sebacina* sp., D: *Amanita;*E, F: *Thelephora afrostuposa*; G: *Thelephorales* ; H : *Russula congoana* I: *Thelephora capitata* J : *Scleroderma* sp , *Amanita* sp*;* K: *Inocybe* sp; L: *Lactifluus acrissimus.* **Scale bars**: A-C-F-I-J-K: 0.35 mm; B-E-G-H-L: 0.1mm; D: 3 mm

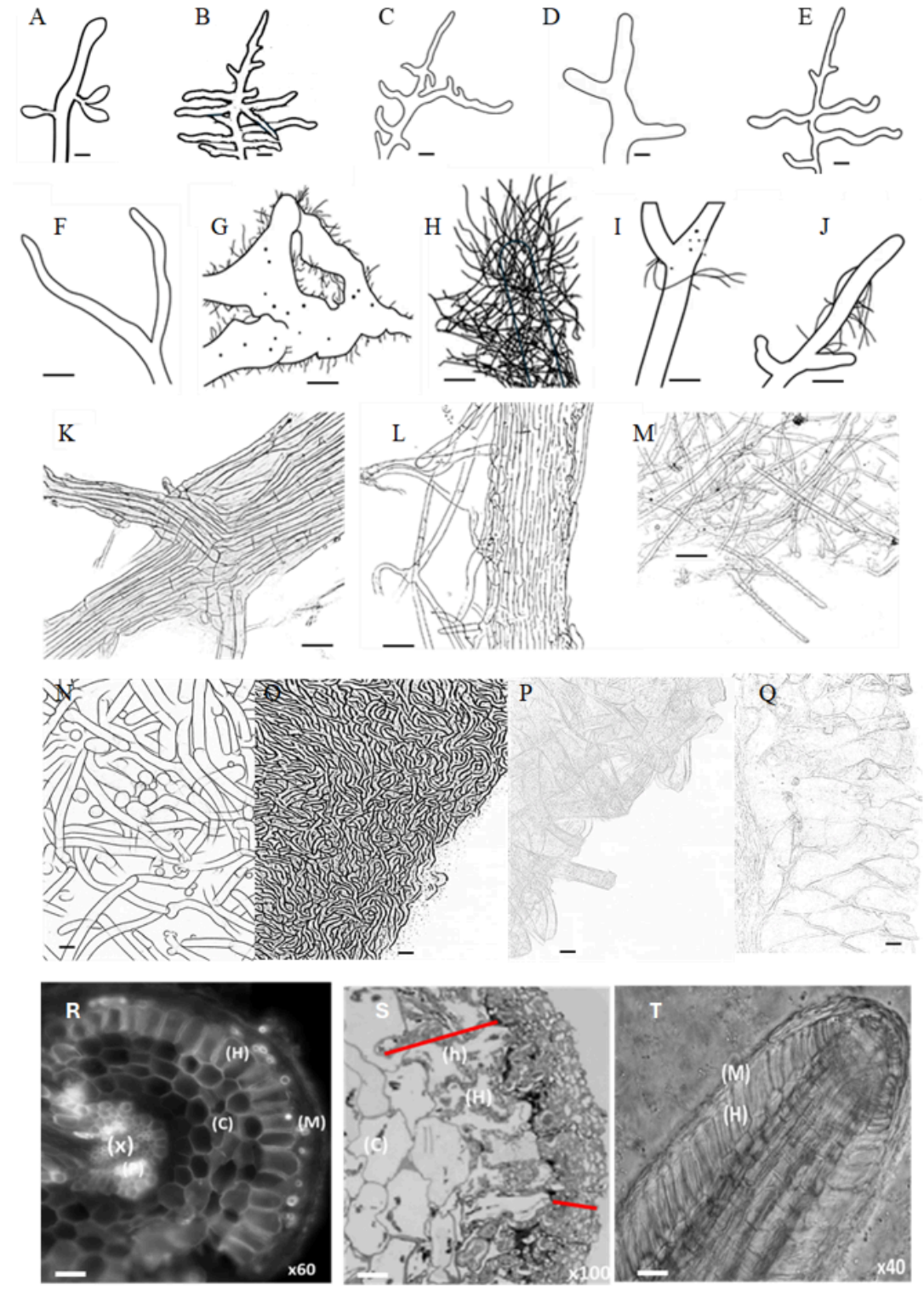


Figure 3

Morphological and anatomical characters used to describe ectomycorrhizal morphotypes associated with *Isoberlinia doka*.

Ramification types: A: simple, B: monopodial-pyramidal, C: monopodial-pinnate.
Unramified ends: D: bent, E: straight, F: sinuous. **Rhizomorph morphology**: G: smooth margins, H: hairy

margins, I: interconnected filaments. Rhizomorph attachment to mantle: J: hyphal fans, K: restricted point, L: flat-angle emergence. **Rhizomorph internal organization**: M: compact undifferentiated hyphae, N: loosely woven hyphae of uniform diameter, O: distinct individual hyphae. **Outer mantle organization**: P: irregular plectenchymatous, Q: dense labyrinthiform plectenchymatous, R: net-like plectenchymatous, S: pseudoparenchymatous with angular cells. **Internal anatomy**: R: Root cross section view; S: Zoom on mantle and Hartig net; T: Longitudinal section of the mantle view. **Abbreviations**: (M) mantle; (H) Hartig net; (C) cortical cells; (X) xylem; (P) phloem; (m) mantle thickness; (h) Hartig net depth. **Scale bars**: A-E: 0.3 mm; F-J: 0.1 mm; K-M: 10 µm; N-Q: 10µm; R: 20µm; S: 10µm; T: 40µm.

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