

# Secondary growth and exodermal barriers shape local root hydraulics: modelling insights in tomato

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## Abstract

Root water uptake efficiency depends on root system architecture and anatomical features of individual root segments. Beyond cell wall, membrane, and plasmodesmata hydraulic properties, root anatomy critically influences profiles of radial conductivity and axial conductance. While these structural factors have been well-characterized in monocotyledons, their role in dicotyledons, where developmental anatomy, secondary growth, and hydrophobic barrier dynamics differ, remains poorly understood. Here, we integrate structural and functional models to assess how dicotyledon-specific anatomy, hydrophobic depositions (suberin/lignin in exo-/endodermis), and aquaporin contribution influence root hydraulics. Using tomato (*Solanum lycopersicum* L., cv. Moneymaker) as a dicotyledon model, our simulations show that:

- Exodermal suberin has negligible effects on radial conductivity when an impermeable lignin cap is present, and exodermal barriers are less effective than endodermal ones.
- Secondary growth and dicotyledon-specific anatomy are essential for sustaining high axial conductance, ensuring efficient water uptake across soil profiles and maintaining root system hydraulic conductance.

**Keywords:** root anatomy, hydraulic conductivity, secondary growth, modelling, tomato

## 1. Introduction

In response to the challenges imposed by changing climate conditions and the need for sustainable food production, there is a growing focus on the development of water-efficient and climate-robust crops (Tebaldi and Lobell 2008). In that purpose, root systems are increasingly recognized as crucial breeding targets (De Dorlodot et al. 2007), as they mediate plant interactions with soil, water, and nutrients, key factors under changing climate conditions (Lynch 1995, Uga 2021). A comprehensive understanding of root system architecture and hydraulics, together referred to as root hydraulic architecture, is essential for identifying local strategies that optimize water uptake (Maurel and Nacry 2020).

The efficiency of water uptake by plants is closely tied to their root system architectures (RSAs) and the anatomical features of individual root segments (Doussan 1998, Heymans

et al. 2020). The root architecture defines the position of roots in the soil, affecting their access to water and nutrients, as well as their connectivity to one another (Lobet et al. 2014). Root anatomy describes the geometry of root tissues from the cell scale. It strongly influences root hydraulic properties at the root segment scale, hereafter termed 'local', such as the radial conductivity ( $k_r$ ) and specific axial hydraulic conductance ( $k_x$ ) (Heymans et al. 2020, Lynch et al. 2021). It is worth reminding that the hydraulic conductance describes total water flow capacity and scales with root size, whereas conductivity (normalized by surface area) and specific conductance (normalized by length) are size-independent measures that allow comparison across roots. Cell-scale features, such as the deposition of hydrophobic barriers (Wang et al. 2019) or aerenchyma formation (Fan et al. 2007) also have a strong influence on the local hydraulic properties. The integration of both root structural

(architecture, anatomy) and functional (hydraulic conductivity) properties is needed to better estimate the water uptake capacities of specific plants (Passot et al. 2019).

To understand water uptake processes, we first need to define water uptake in roots, particularly from the soil to the xylem. The radial movement of water through the cell anatomical network is commonly divided in two parallel pathways; the apoplastic pathway (through cell walls), and the cell-to-cell pathway ('transmembrane' across cell plasma membranes, and 'symplastic' through plasmodesmata) (Steudle 2000). Apoplastic flow can be blocked by the formation of hydrophobic barriers, with substances such as lignin (e.g. Casparian strips, lignin cap), while suberin hinders transmembrane flow (when forming waxy secondary walls covering the cell plasma membrane), in the endodermis and/or the exodermis (for reviews, see Enstone et al. 2002, Geldner 2013, for lignin cap, see Manzano et al. 2025). Furthermore, water flow in the cell-to-cell pathway is regulated by the aperture of plasmodesmata (see Bayer and Benítez-Alfonso 2024 for review) and the permeability of cell membranes via aquaporins (Vandeleur et al. 2009, Couvreur et al. 2018). All of these cell-scale features and their interplay highly influence  $k_r$  (see Heymans 2022 for sensitivity analysis).

Structural anatomical features also influence  $k_r$  and define  $k_x$ . Notably,  $k_r$  is highly influenced by cortex width, or stele/cortex area ratio (Chimungu et al. 2014, Heymans et al. 2020), while  $k_x$  is dependent on the number and size of xylem vessels (Clément et al. 2022). It is worth mentioning that experimental measurements of  $k_x$  can deviate from simple computation of Hagen–Poiseuille equation because of finite xylem vessel lengths and increased resistance due to border pits (Choat et al. 2008, Boursiac et al. 2022, Strock et al. 2024).

These effects of structural anatomical features on  $k_r$  and  $k_x$  have been extensively studied in monocotyledons like maize, wheat or wheatgrass (Sanderson et al. 1988, Heymans et al. 2020, 2021) and small dicotyledons like *Arabidopsis* or lupin (Meunier et al. 2018, Boursiac et al. 2022). However, there is still a lack of knowledge on the influence of specific anatomical traits on hydraulic properties in woody plants (like tomato or grapevine), where clear anatomical differences must impact root hydraulics (Strock and Lynch 2020).

First, monocotyledons and dicotyledons root anatomies differ in terms of tissues and layers organization, most notably in the patterning of metaxylem and phloem (Lynch et al. 2021, Clément et al. 2022). In addition, dicot roots can undergo secondary growth (even in annual plants), which consist in a radial expansion due to the development of the stele, and an increase in parenchyma, phloem and xylem vessels, leading to an overall increasing diameter. This could have a significant effect on the radial hydraulic conductivity (Strock and Lynch 2020).

Second, the timing and the type of hydrophobic barriers differ between species. In numerous plant species, the endodermis serves as a selective barrier, with the deposition of lignin (Casparian strips) and suberin lamellae (Geldner 2013). Beyond the endodermis, outer tissues such as the multiseriate cortical sclerenchyma and exodermis can also develop hydrophobic barriers, contributing to the regulation of water and nutrient transport (Schneider et al. 2021, Zhu et al. 2025).

While *Arabidopsis thaliana* has long been established as a model species for dicotyledonous plants, its lack of exodermal barrier formation which is a feature present in more than 90% of angiosperms, limits its representativeness in studies of root water uptake. Consequently, there is a need to identify alternative model species that more accurately reflect the diversity of hydrophobic barrier structures in angiosperms. In tomato, recent advances in functional genetics unravelled the formation of suberin and lignin in exodermis, highlighting it as a promising candidate for such investigations. More specifically, Manzano et al. (2025) showed that in tomato, exodermis forms a polar lignin cap starting from 0.4 cm from the tip and with maximum lignification at 8 cm from the tip. Cantó-Pastor et al. (2024) showed that suberin deposition do not happen in endodermis, but well in exodermis, first as 'patchy suberized' starting between 3 and 5 days, then as 'continuously suberized' after 5 days. Both papers linked these apoplastic barrier depositions with water stress adaptation, by using tracers, or by investigating the expression in different water conditions, respectively.

While this progress is substantial, the interaction between these hydrophobic depositions, secondary growth formation, and specific anatomical patterns must be studied to fully capture radial water dynamics in roots. However, such investigations are technically challenging experimentally (Passot et al. 2019, De Swaef et al. 2022).

Over the past decade, the scientific community has developed so-called *Structural Functional Plant Models* (FSFM). *Structural* models represent the plant structure at different scales: root anatomy (e.g. Heymans et al. 2020, Sidhu et al. 2023), root system architecture (e.g. Pagès et al. 2014, Schnepf et al. 2018) or whole plant system (Zhou et al. 2020). These structural models are designed to be coupled to *functional* models, notably hydraulic models, to simulate water uptake at the anatomical scale (Couvreur et al. 2018), in a root system hydraulic architecture (Doussan 1998, Meunier et al. 2020) or in the whole plant framework (Giraud et al. 2023). They have been used to investigate the interaction between root architectural and/or anatomical traits and water uptake (e.g. Zarebanadkouki et al. 2016, Meunier et al. 2018, Boursiac et al. 2022, Heymans 2022, McLaughlin et al. 2024, Yu et al. 2024). Notably, these models allow to investigate the influence of hydraulic conductivities of root segments on root system architecture hydraulic properties. Such properties include root system hydraulic conductance  $K_{rs}$  and standard uptake fractions *SUF*, which are the relative contribution of each segment to the total water uptake in homogenous soil, from Couvreur et al. (2012).

In this work, we used experimental data and computational models of roots—the cross-section structural generator GRANAR (Heymans et al. 2020) linked to the hydraulic solver MECHA (Couvreur et al. 2018)—to study how combined changes in root anatomy (including secondary growth), hydrophobic depositions levels (suberin and/or lignin in endo- and/or exodermis) and aquaporin contribution to membrane conductivity influence the root hydraulic properties in tomato (*Solanum lycopersicum* L., var. MoneyMaker). We also investigated to what extend these root hydraulic conductivities can shape root system hydraulic conductance and

standard uptake fractions. For convenience, we limited the analysis to root anatomies before periderm formation, which was never observed in our cross-sections.

## 2. Methods

### 2.1 Plant growth and sample preparation

40 tomato (*Solanum lycopersicum* var. Moneymaker) plants were grown in UCLouvain greenhouses (Louvain-la-Neuve, Belgium) from October 12 to 11 November 2022, in an aeroponic system at 24°C. Seeds were directly dropped in small PVC tubes composed of a plastic web as a floor and filled with a mix of perlite and vermiculite (1:1) to allow the plant to stabilize. The tubes were fixed in expanded polyester plates (10 × 50 cm) and mounted on top of the aeroponic system (de Dorlodot et al. 2005). Watering was done by spraying Hoagland nutrient solution for 5 s every 3 minutes. Plants were collected after 30 days of growth. We selected the 9 healthier plants (that presented secondary growth) and harvested their root systems. Tap roots were cleaned from lateral roots and stored in WEG (water–ethanol–glycerol 3:3:2) as recommended by Kitin et al. (2020).

### 2.2 Microscopy

The roots stored in WEG were divided into 4–6 segments of length 1–3 cm (depending on the position along the root: smaller segments near the tip and at secondary growth formation, longer segments for visually consistent intermediate phase), in which anatomy was considered as consistent to replicate microscopy observations. The length of each segment of each plant as well as the length of the whole root were carefully measured. Based on previous manipulations and data, we simplified the root growth rate by simply taking the mean through the entire growth period. Since all roots were not the same length but the same age, we calculated the length/age ratio for each root. The position of each segment was then expressed in days instead of centimetres.

Root segments were deposited in small moulds and embedded in an agarose solution (5% w/v in distilled water, at 40°C), following the Atkinson and Wells (2017) protocol. It is worth noting that samples must never be dry, otherwise the cells will shrink, so we advise to always keep the samples in 50% glycerol. For very thin root segments, we reduced the concentration of agarose to 3%.

Once the agarose became solid, cross-sections were made manually, as thin as possible (~200 µm) using a simple double edge razor blade. We advise keeping the samples in drops of 50% glycerol when cutting. Cross-sections were deposited in Petri dishes in a drop of distilled water (or 50% glycerol for fluorol yellow staining). Then cross-sections were stained with a drop of toluidine blue O (0.05% w/v in distilled water) for a few seconds, then carefully rinsed with distilled water, and observed in microscope under white light (Eclipse E400, NIKON).

For three roots out of the nine, additional staining was applied to reveal apoplastic barriers in every root segment. To reveal lignin, the cross-sections were stained in berberine hemisulfate (0.1% w/v in distilled water) for 1 h, then rinsed with distilled water and counter-stained in aniline blue (0.5% w/v in distilled water) for 30', then rinsed in distilled water (Brundrett et al. 1988). To reveal suberin, cross-sections were stained with fluorol

yellow 088 (0.05% in glycerol) for 1 h, then rinsed with 50% glycerol and mounted on 50% glycerol slides (Kitin et al. 2020). For both colourations, microscopical pictures were taken under UV-excitation (with FITC filter) with an Eclipse E400 (NIKON). For each cross-section of each root segment, pictures were taken (Moticam 2000, MOTIC) at ×20 or ×10 zoom.

### 2.3 Anatomical features quantification

Cross-sections pictures were quantified using ImageJ (Schneider et al. 2012) to get data in the form of GRANAR parameters (Heymans et al. 2020). 45 cross-sections were quantified from the 9 different tap roots (21 were classified as 'maturation' phase, 8 as 'intermediate' phase and 16 as 'secondary growth' phase). All images are available at <https://zenodo.org/records/20677866>. The full quantification protocol consists of drawing a few cells for each tissue and measuring their size (in µm<sup>2</sup>) or their width (in µm). For metaxylem, each vessel cell was drawn, because the number of metaxylem vessels is a parameter of GRANAR, and it is obtained by counting the number of measurements. Protoxylem was considered as parenchyma. For parenchyma, phloem and cortex, cells were drawn along one or more radius, to avoid unintentional selection bias. For tissues that are composed of one single cell layer (pericycle, endodermis, exodermis, and epidermis), a representative subset of cells was drawn along the arc of circle of the cell layer. For phloem, pericycle, endodermis, cortex, exodermis and epidermis, the cell width was measured, oriented in the cross-section radius direction.

In secondary growth cross-sections, cambium was classified as phloem sieve tubes. Indeed, it was hard to distinguish the two tissues in our pictures, and we do not aim to do a developmental analysis. We therefore made the hypothesis that phloem and cambium have a similar behaviour in terms of radial hydraulic conductivity. Even if we expect greater axial conductance in phloem than in cambium, we hypothesize that it does not have a significant influence due to the relatively small proportion of cambium compared to phloem and xylem.

GRANAR generates round cells, whereas cells in these tissues appear to be elongated. We therefore chose to generate round cells in GRANAR but with a diameter equal to the width of the measured cells. By doing so, we end up with generated tissues that have more cells (that are round) in total, but with a diameter equal to the width of the measured cells in the direction of the cross-section radius. This choice was motivated by the fact that layer width has tremendous influence on radial water flow while the number of cells per cell layer (and by extension the number of cell walls for apoplastic pathway) have little effect (Heymans et al. 2020). All measurements of all pictures were stored in a database and treated in R (R Core Team 2022) to get a table of the quantifications as GRANAR input parameters per plant and per segment.

### 2.4 Update of GRANAR to generate secondary growth

The codes of GRANAR and the description of the workflow are available on GitHub (<https://github.com/granar/granar>).

It is worth noting that GRANAR present some limitations due to the nature of its implementation. Notably, the switch

from primary growth to secondary growth is not smooth and corresponds to two different code workflows. While in primary growth mode GRANAR explicitly generates xylem blades, in secondary growth mode GRANAR generates randomly placed metaxylem vessels around the centre of the stele, then directly losing the typical blade formation.

## 2.5 Computing water flow in hydraulic anatomies

We computed  $k_r$  and  $k_x$  of every quantified anatomy with MECHA (Couvreur et al. 2018) for 60 scenarios: 6 different deposition levels, and 10 values of contribution of aquaporin to membrane permeability  $k_{AQP}$  [ $\text{cm};\text{d}^{-1}\text{hPa}^{-1}$ ]. The other hydraulic parameters of MECHA were set as default values, coming from literature (Supplemental S4) (see Couvreur et al. 2018).

The different hydrophobic deposition levels are summarized in Table 1. This choice was supported by our own observations, as well as results from Cantó-Pastor et al. (2024) and Manzano et al. (2025). Monocot deposition levels are the standard ones for maize from Doussan (1998) and Heymans et al. (2020). The test values of  $k_{AQP}$  range from  $1\text{E}-04$  and  $1\text{E}-03 \text{ cm d}^{-1} \text{ hPa}^{-1}$ , to basically explore its influence around the mean value from maize;  $4.3\text{E}-04 \text{ cm d}^{-1} \text{ hPa}^{-1}$  (Ehlert et al. 2009, Couvreur et al. 2018). It is useful to note that such units [ $\text{cm d}^{-1} \text{ hPa}^{-1}$ ] were chosen for ease of reading instead of standard [ $\text{m s}^{-1} \text{ MPa}^{-1}$ ].

## 2.6 Statistical analysis of the influence of anatomical features on hydraulic properties

### 2.6.1 Effect of age, radius, aquaporins and hydrophobic barriers on radial conductivity

A Box–Cox transformation (Box and Cox 1964) of parameter  $\lambda = -0.4242$  was applied to  $k_r$  values, and improved the normality of the data, going from  $P < 2.2 \times 10^{-16}$  to  $P = 1.97 \times 10^{-10}$  under Shapiro–Wilk test. While the

**Table 1** Definition of hydrophobic depositions, for tomato and contrast comparison.

| Type                                    | Name                 | Description                                            |
|-----------------------------------------|----------------------|--------------------------------------------------------|
| Tomato<br>(experimental)                | <i>En.CS</i>         | Endodermal Casparian strips                            |
|                                         | <i>En.CS-Ex.LC</i>   | Endodermis Casparian strips and exodermal lignin cap   |
|                                         | <i>En.CS-Ex.Sub</i>  | Endodermis Casparian strips and exodermal suberization |
| Contrast<br>Comparison<br>(theoretical) | <i>En.Sub</i>        | Endodermal suberization                                |
|                                         | <i>En.Sub-Ex.CS</i>  | Endodermal suberization and exodermal Casparian strips |
|                                         | <i>En.Sub-Ex.Sub</i> | Endo and exodermal suberization                        |

Shapiro–Wilk test statistic remained significant, the high explanatory power of the model, combined with visual validation of predicted versus observed values, supports the robustness of our approach.

### 2.6.2 Effect of lignin permeability on radial conductivity

Estimated marginal means were calculated for each apoplastic deposition type within the two levels of lignin permeability using the *emmeans* package. Pairwise comparisons of estimated marginal means were performed to test for differences between all types of apoplastic depositions separately for each level of lignin permeability.

### 2.6.3 Effect of age and radius on specific axial conductance

A Box–Cox transformation of parameter  $\lambda = -0.141$  was applied to  $k_x$  data, to force normality, going from  $P = 0.001574$  using neperian logarithm transformation to  $P = 0.00315$  using Box–Cox (under Shapiro–Wilk test).

The linear regression model has the following equation:

$$k_{xBC}^* = -231.99 + 53.79 \cdot \text{radius} + 3.79 \cdot \text{age} \quad (1)$$

$$k_x^* = (-0.141 k_{xBC}^* + 1)^{-\frac{1}{0.141}}$$

where  $k_x^*$  [ $\text{m}^4 \text{ s}^{-1} \text{ MPa}^{-1}$ ] is the estimated specific axial conductance, after transformation with Box–Cox technique.

## 2.7 Root system hydraulic architectures

10 virtual root systems were simulated using the CRootBox shiny app ([plantmodelling.shinyapps.io/shinyRootBox/](https://plantmodelling.shinyapps.io/shinyRootBox/)) (Schneppf et al. 2018). We used the *Arabidopsis thaliana* preset and modified some parameters based on our own data (see Supplemental S3), and by setting the deviation of these parameters to 30%. *Tropism parameter 1* was set to 1.5 for lateral roots (type 2).

We defined 4 *in silico* mutants by changing radius,  $k_r$  and  $k_x$  and *s* of each RSA segments (Table 2).

- *WT*: an *in silico* wild type of tomato.
- Hydrophobic barriers set successively as *En.CS-Ex.LC* than *Ex.CS-Ex.Sub* (starting at respectively 0 and 7 days).
- Root radius of each segment applied as a function of the root segment age.
- $k_{AQP}$  set constant at  $5 \times 10^{-4} \text{ cm} \cdot \text{hPa}^{-1} \cdot \text{d}^{-1}$ .
- $k_r$  and  $k_x$  of each segment calculated using the regression functions.
- *aqp*: an *in silico* mutant of aquaporins.
- Hydrophobic barriers set successively as *En.CS-Ex.LC* than *Ex.CS-Ex.Sub* (starting at respectively 0 and 7 days).
- Root radius of each segment applied as a function of the root segment age.
- $k_{AQP}$  set as linearly decreasing from  $5 \times 10^{-4}$  to  $1 \times 10^{-16} \text{ cm} \cdot \text{hPa}^{-1} \cdot \text{d}^{-1}$  along root age.
- $k_r$  and  $k_x$  of each segment calculated using the regression functions.
- *sub*: an *in silico* mutant of suberin.

**Table 2** Definition of the *in silico* mutants.

| Name          | Endodermal deposition | Exodermal deposition        | Secondary growth | Aquaporin contribution |
|---------------|-----------------------|-----------------------------|------------------|------------------------|
| <i>WT</i>     | Casparian strips      | Lignin cap and suberization | Yes              | Constant               |
| <i>Aqp</i>    | Casparian strips      | Lignin cap and suberization | Yes              | <b>Decreasing</b>      |
| <i>Sub</i>    | Casparian strips      | <b>Lignin cap</b>           | Yes              | Constant               |
| <i>sg-sub</i> | Casparian strips      | <b>Lignin cap</b>           | <b>No</b>        | Constant               |

- Hydrophobic barriers fixed to *En.CS-Ex.LC* for all root segments.
- Root radius of each segment applied as a function of the root segment age.
- kAQP set constant at  $5 \times 10^{-4} \text{ cm} \cdot \text{hPa}^{-1} \cdot \text{d}^{-1}$ .
- $k_r$  and  $k_x$  of each segment calculated using the regression functions.
- *sg-sub*: an *in silico* counter-witness with no secondary growth and no suberin.
  - Hydrophobic barriers fixed to *En.CS-Ex.LC* for all root segments.
  - Root radius fixed to 0.198 mm for all root segments.
  - kAQP fixed at  $5 \times 10^{-4} \text{ cm} \cdot \text{hPa}^{-1} \cdot \text{d}^{-1}$ .
  - $k_r$  and  $k_x$  of each segment calculated using the regression functions.

As radius and  $k_x$  regression functions are exponential, we set maximum radius at 2 mm and maximum  $k_r$  at  $2\text{E-}10 \text{ m}^3\text{MPa}^{-1}\text{s}^{-1}$ .

We implemented a framework to loop the computation of root system hydraulic architecture (RSHA) for each desired timestep of each desired RSA. For each iteration, MARSHAL (Meunier et al. 2020) was run under homogeneous soil water potential, with boundary collar water potential at  $-15 \text{ kPa}$ . Root system hydraulic conductance  $K_{rs}$  and standard uptake fractions *SUF* were evaluated every 10 days from 10- to 60-day-old RSA.

Statistics on  $K_{rs}$  and *SUF* were made using linear regressions and estimated marginal means. Normality of the data was evaluated by looking at the residuals of the linear regressions.

## 2.8 Data analysis and packages

All statistical analysis was performed using R software (R Core Team 2022).

The function ‘lm’ from the ‘stat’ package was used to build linear regressions. The function ‘cor’ from the ‘stat’ package was used to compute Pearson correlation coefficients. The function ‘shapiro.test’ from the ‘stat’ package was used to perform a Shapiro–Wilk normality test. The function ‘boxcox’ from the package ‘MASS’ was used to estimate  $\lambda$  parameters of Box–Cox transformations. The function ‘emmeans’ from ‘emmeans’ package was used to compute estimated marginal means. Graphs were produced using ‘ggplot2’, ‘ggthemes’ and ‘vidiris’ packages.

## 2.9 Use of artificial intelligence

Some portions of text were reformulated with AI tools, namely LeChat (<https://mistral.ai/>) and ChatGPT (<https://openai.com/>), to enhance clarity and coherence while preserving scientific

consistency. Here is an example of prompt used to format results from bullet points to text: ‘Act as a scientist specialized in plant science. Write or rephrase what I give you in a scientific way. Be precise and use a scientific language. Avoid complex and pompous formulations. Generate only the text, nothing more.’ All generated content was critically edited and reviewed.

## 3. Results

### 3.1 Quantification of anatomical features

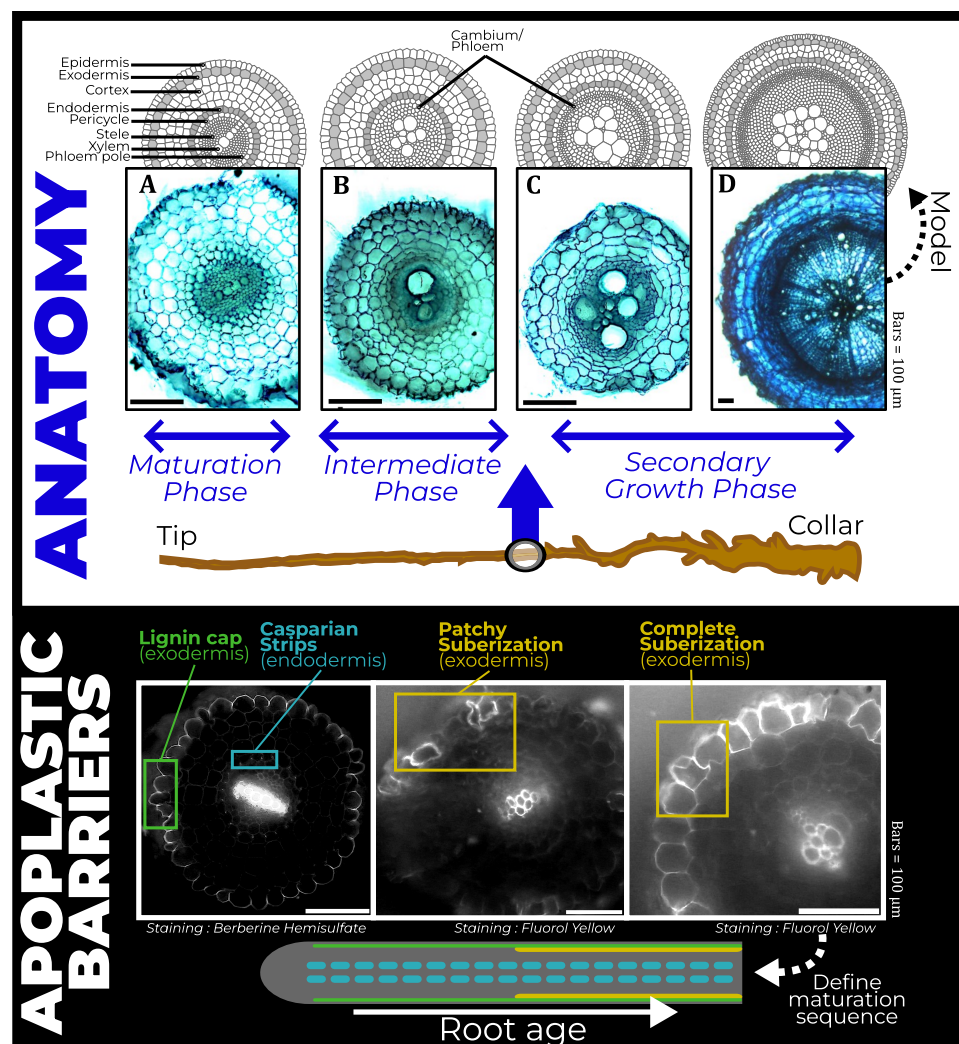
We analysed anatomical features of 45 cross-sections from nine main roots of 30-day-old tomato plants (*Solanum lycopersicum* L. var. *MoneyMaker*). From these observations, we classified tomato root anatomy into 3 phases: *maturation*, *intermediate* and *secondary growth* (respectively 21, 8, and 16 cross-sections). Note that the meristematic and elongation zones were too short to be characterized by our methods ( $<5 \text{ mm}$  from the tip). Because roots did not have the same length, we choose to express the localization of measurements in terms of days of growth instead of distance to tip.

The first phase is the *maturation* stage, which expanded from 0 to 5–7 days (picture A in upper panel of Fig. 1). It was characterized by two blades of xylem across the stele. Xylem and phloem were organized in poles, with two blades of xylem sectioning the inner stele. The cortical cylinder was composed of one layer of endodermis, 3–4 cortex cell layers, one layer of exodermis and one layer of epidermis.

The second phase, that we called *intermediate*, was characterized by the development of two new metaxylem files on each side of the xylem blades and expands from 5–7 to  $\sim 25$  days (picture B in upper panel of Fig. 1). This stage was also characterized by an average of 3 cortex cell layers. At the end of this phase, the cambium/phloem began to form a ring around the inner stele, slightly introducing secondary growth.

Finally, the *secondary growth* stage (pictures C and D in Fig. 1), starting approximately after 25 days, consisted of further exponential growth of the stele, with more parenchyma and xylem vessels, together with a large increase of xylem vessel size (Supplemental S1). Xylem patterning switched from blades to a heterogeneous disseminated placement in the inner stele (which is the main difference between pictures B and C in Fig. 1). The phloem tissue multiplied, expanding into multiple layers, ranging from 2 to 5, while concurrently, the cortex was reduced to 2 layers (picture D in Fig. 1). It is worth noting that we did not observe any periderm formation in the cross-sections.

The root radius along its longitudinal axis followed two trends: first it could be approximated as a constant ( $0.2 \pm 0.03 \text{ mm}$ ) until the root segment age was 25 days



**Figure 1** Quantification of tomato root anatomy features and hydrophobic barriers. Tomato roots (middle) were used to characterize and model anatomy (top) and hydrophobic barriers (bottom). The observed anatomy (top) was monitored and quantified to update and train a secondary growth module of the structural model GRANAR. Lignin and suberin depositions were observed in endodermis and exodermis to define the sequence of apoplastic barriers formation.

( $P < 10^{-3}$ ). After, it increased with age, so we built a linear regression of root radius as a function of root age with a Box-Cox transformation ( $P < 10^{-4}$ ;  $R^2 = 0.729$ ). This function was then used to predict the root radius of segments as a relation of their age.

Among the anatomical parameters, most of them showed a Pearson correlation coefficient (denoted  $r$ ) equal or higher than 0.5 with the root segment age, except in the cell diameter of phloem sieve tubes ( $r = 0.42$ ) and cell diameter of pericycle ( $r = 0.35$ ), which both displayed substantial variability regardless of root segment age. Notably, a negative correlation ( $r = -0.69$ ) was spotted between the number of cortex layers and age, illustrating a reduction from 4 layers to 2 layers along the longitudinal axis of the root.

The strongest association between anatomical features and the age of the root segment was the diameter of xylem vessels ( $r = 0.96$ ), the maximum size of xylem ( $r = 0.95$ ) and the number of phloem layers ( $r = 0.92$ ). In addition, a positive correlation between the root radius and root age was observed ( $r = 0.6$ ) independently from the number of cortex layers

( $r = -0.29$ ) and the proportion of phloem ( $r = 0.4$ ). The root radius was best correlated to the whole stele diameter ( $r = 0.99$ ), the diameter of stele parenchyma cells ( $r = 0.95$ ) and the number of metaxylem vessels ( $r = 0.94$ ).

### 3.2 Apoplastic barriers monitoring

To access the types of apoplastic barriers in the roots, we monitored qualitatively lignin and suberin deposition (lower panel in Fig. 1). First, we observed the formation of Casparian strips in the endodermis starting at the maturation zone of the root, and the deposition of lignin in the outer-periclinal and anticlinal cell walls of the exodermis, forming a polarly localized lignin cap. We defined these two layers as deposition level *En.CS-Ex.LC* (for Endodermal Casparian Strips—Exodermal Lignin Cap). Next, in the intermediate and secondary growth zones, the exodermis showed a complete suberin deposition, while the endodermis still showed Casparian strips; we defined this second deposition level as *En.CS-Ex.Sub* (for Endodermal Casparian Strips—Exodermal Suberization). It is

worth noting that *En.CS-Ex.Sub* includes both lignin cap and suberin in exodermis. We did not explicitly name it in the scenario names to avoid overcrowding them with acronyms.

The timings of our observations are reported in [Supplemental S5](#). Because this analysis was not designed to map apoplastic barrier formation along the root, the limited number of replicates does not allow a precise characterization of its spatial progression. Nonetheless, these observations are in the range reported in the literature ([Cantó-Pastor et al. 2024](#), [Carvajal et al. 2025](#), [Manzano et al. 2025](#)), with differences likely arising from variation in the genotype, conditions and growth technique.

### 3.3 Development of a secondary growth module in GRANAR and estimation of local hydraulic properties

The updated version of GRANAR simulates root transverse anatomies that can present secondary growth (on top of [Fig. 1](#)). A complete description and the list of parameters are presented in [Supplemental S2](#). The secondary growth module is a set of R functions that were directly integrated to the main code of GRANAR. With the updated version, users can choose to generate dicotyledon root cross-sections in primary growth stage or in secondary growth stage. Examples of simulated root cross-sections with secondary growth are shown in the top box of [Fig. 1](#). For the 40 quantified root anatomies, the measurements were translated into GRANAR parameters, and a virtual root anatomy was generated. Then, we used MECHA to compute  $k_r$  and  $k_x$  on each of the virtual anatomy, for 10 values of kAQP and 6 different hydrophobic deposition types ([Table 1](#)).

### 3.4 Investigation of the effect of anatomical features on radial conductivity

We first analysed the correlations between  $k_r$  and anatomical quantifications in the case of endodermal Casparian strips (*En.LC*) and mean kAQP value ( $4 \times 10^{-4} \text{ cm hPa}^{-1} \text{ d}^{-1}$ ).  $k_r$  is negatively correlated ( $r \leq -0.5$ ) with most anatomical features except maximum xylem size, xylem cell diameter, phloem layers number, phloem proportion and cortex layers number. Notably,  $k_r$  is highly negatively correlated with cortex cell diameter ( $r = -0.76$ ), radius ( $r = -0.75$ ) and stele layer diameter ( $r = -0.65$ ).

We made a linear mixed model to explain the variance of  $k_r$  by changes in age, radius, aquaporin contribution to membrane permeability, and hydrophobic deposition types. All variables have a significant effect on  $k_r$  (at  $P < 10^{-3}$ ). The generated linear model explains 92.4% of the variance of  $k_r$  and was used as predictive model for the next sections.

### 3.5 Specific effects of hydrophobic barriers deposition on radial conductivity

To assess the influence of hydrophobic barriers on  $k_r$ , we performed a comparison of the mean of  $k_r$  while fixing aquaporin contribution at its average value ( $4 \times 10^{-4} \text{ cm d}^{-1} \text{ hPa}^{-1}$ ). In addition to our observed scenarios *En.CS-Ex.LC* and *En.CS-Ex.Sub*, we included a scenario with endodermal Casparian strip and nothing in exodermis (*En.CS*), as well as three artificial, unrealistic scenarios with suberin in endodermis (*En.Sub*, *En.Sub-Ex.LC*, *En.Sub-Ex.Sub*), to evaluate the theoretical impact of having or

not suberin in endodermis. All simulated scenarios are summarized in [Table 1](#).

Our analysis showed that the presence of an impermeable exodermal lignin cap has no significant difference in terms of  $k_r$  compared to exodermal suberin ([Fig. 2D](#)). Even combined with either an endodermal Casparian strip or suberized endodermis, the resulting  $k_r$  values were not significantly different from one another. In contrast, a clear reduction in conductivity emerged when an exodermal lignin cap was introduced relative to the case where there was no hydrophobic barrier in this cell layer. Specifically, adding a lignin cap to exodermal cells alongside an endodermal Casparian strip reduced  $k_r$  by 20.35%, whereas full suberization of the endodermis produced a stronger effect, lowering  $k_r$  by 45%, compared to a scenario with only an endodermal Casparian strip.

When scaling these values to the level of the whole cross-section, radial conductance ( $K_r$ ) was calculated by multiplying the computed  $k_r$  of the cross-section by the perimeter.  $K_r$  then represents a flow of water per root length, per pressure unit and per time unit. Although the radial conductivity  $k_r$  drops (in average for the 3 deposition types) by 15.3% between 15–25 and 25–30 days periods due to secondary growth, the radial conductance per unit length ( $K_r$ ) consistently increased by 18.9% between 15–25 and 25–30 days because of the expanding root perimeter ([Fig. 2B](#)).

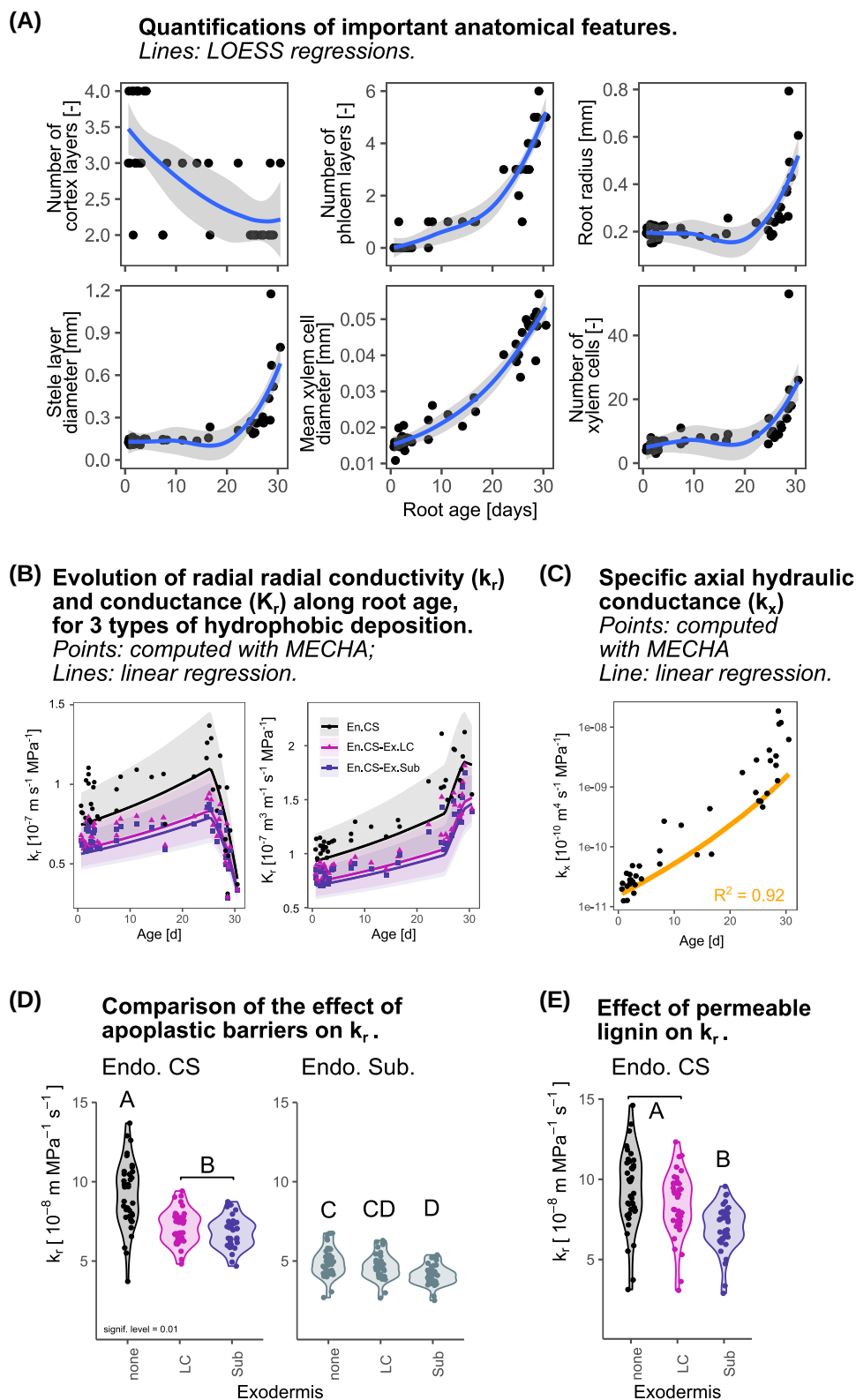
Since lignin is a highly heterogenous polymer, its precise structure is not known. While its link with cellulose is supposed hydrophobic, nanopores in the network could theoretically still let water pass. For consistency, we ran simulations with a lignin permeability of  $4.3 \times 10^{-7} \text{ cm}^2 \text{ d}^{-1} \text{ hPa}^{-1}$ , that was estimated by [Couvreur et al. \(2018\)](#), based on 1 nm pores ([Fig. 2E](#)). At mean aquaporin contribution ( $4 \times 10^{-4} \text{ cm d}^{-1} \text{ hPa}^{-1}$ ), the influence of lignin permeability significantly affects  $k_r$  ( $P = 0.0027$ ). Pairwise comparison of estimated marginal means shows no significant difference between nothing in exodermis and a permeable lignin cap in exodermis ( $P = 0.055$ ). In contrast, there is a significant difference between permeable lignin cap in exodermis and suberin deposition in exodermis ( $P = 0.0016$ ) ([Fig. 2E](#)). In that case, suberin in exodermis decrease  $k_r$  by 17.1% compared to permeable lignin cap in exodermis. For simplicity, we considered lignin cap as impermeable for the rest of this work.

### 3.6 Effect of radius and age on specific axial conductance

$k_x$  is highly correlated with xylem vessel number and size due to the Hagen–Poiseuille relation (respectively  $r = 0.91$  and  $r = 0.65$ ) and is also highly correlated with root radius ( $r = 0.91$ ). Since xylem cell diameter is highly correlated with root age ( $r = 0.96$ ) and xylem cell number is highly correlated with root radius ( $r = 0.94$ ), we constructed a linear model to predict  $k_x$  in function of root segment age and radius ( $P < 10^{-3}$ ;  $R^2 = 0.92$ ) ([Table 3](#)) ([Fig. 2C](#)).

### 3.7 Application: simulations of root system hydraulic architecture

To illustrate the effect of hydrophobic barriers and secondary growth on root system water uptake, we defined 4 *in silico*



**Figure 2** Quantitative analysis of root anatomical features, hydraulic properties, and the impact of hydrophobic depositions. (A) Quantifications of key anatomical features over root age. Data points represent measured values, while lines indicate LOESS regressions for visualization. (B) Evolution of radial conductivity ( $k_r$ ) and conductance ( $K_r$ ) along root age for three types of hydrophobic deposition. (C) Specific axial hydraulic conductance ( $k_x$ ) computed with MECHA following Hagen–Poiseuille equation (points) and predicted with linear regression (line). (D) Comparison of the effect of hydrophobic barriers (LC for lignin cap and Sub for suberin) on radial conductivity ( $k_r$ ) for endodermal casparian strips (Endo. CS) and endodermal suberin (Endo. Sub). (E) Effect of permeable lignin on radial conductivity ( $k_r$ ), for the 3 deposition levels of exodermal apoplastic barriers (none, Lignin Cap and Suberin).

**Table 3** Summary of the linear regression of specific axial conductance in function of age and radius.

| Variable               | Estimate  | Std. error | T value | P value  |
|------------------------|-----------|------------|---------|----------|
| (Intercept)            | −231.9857 | 5.0813     | −45.655 | <10−4    |
| Radius                 | 53.7887   | 22.3322    | 2.409   | 2.10E−02 |
| Age                    | 3.7873    | 0.2473     | 15.313  | <10−4    |
| Adjusted $R^2 = 0.919$ |           |            |         |          |

mutants; a wild type (*WT*), an aquaporin mutant (*aqp*), a suberin deficiency mutant (*sub*) and a combined secondary growth deficient—suberin deficient mutant (*sg-sub*) (Table 2). We assumed no interactions with other cell hydraulic or root anatomical traits, and we set lignin as an impermeable apoplastic barrier. We defined the timing of formation of apoplastic barriers at 0 and 7 days, for exodermal lignin cap and exodermal suberization, respectively. This assumption was based on qualitative measurements (Supplemental S5).

We first generated 10 tomato-like simplistic RSA of 60 days old with CRootBox (Schnepf et al. 2018). Then for each *in silico* mutant,  $k_r$  and  $k_x$  were computed for each root segment of the 10 RSA. It is worth noting that the axial conductance remains low for *sg-sub*, since there is no increase of diameter, compared to the *WT* and the other mutants.

For all *in silico* mutants, we computed the relative contribution of each segment to the total water uptake (*SUF*; Standard Uptake Fractions from Couvreur et al. 2012, under uniform soil water potential), hereafter named ‘relative water uptake’, and the root system hydraulic conductance  $K_{rs}$  [ $\text{m}^3\text{MPa}^{-1}\text{s}^{-1}$ ] with the root system hydraulic architecture solver MARSHAL (Meunier et al. 2020) every 10 days.

### 3.7.1 Root system hydraulic conductance

Statistical analyses showed no differences in  $K_{rs}$  neither between *aqp* mutant and *WT* ( $P = 0.667$  at 30 days and  $P = 0.21$  at 60 days) nor between *sub* mutant and *WT* ( $P = 0.768$  at 30 days and  $P = 0.253$  at 60 days). In contrast, *sg-sub* mutant showed a reduction of  $K_{rs}$  compared to *WT* (40.3% at 30 days, 64.5% at 60 days, both at  $P < 10^{-4}$ ) (Fig. 3B). A simple linear regression model incorporating RSA age, mutant type and their interaction effectively described  $K_{rs}$  variability ( $R^2 = 0.94$ ). Under  $P < 0.05$ , the model showed no significant effect of *aqp* and *sub* compared to *WT*, and no significant effect of the interactions age-*sub*. By contrast, the effects of *sg-sub*, age, age-*aqp* interaction and age-*sg-sub* interaction were significative. These results show that the absence of secondary growth highly reduce  $K_{rs}$ , and the latter can be predicted linearly in function of the age.

### 3.7.2 Relative water uptake

No significant difference was detected in the whole water uptake profile between *WT* and *sg-sub* (under Kolmogorov–Smirnov test;  $P = 0.952$  at 30 days old and  $P = 0.994$  at 60 days old) (Fig. 3C). Despite the absence of global differences, local shifts of relative water uptake at different soil layers were observed between *WT* and *sg-sub* at specific soil layers, at

significance level of  $P < 5 \times 10^{-3}$ . For 30 days-old RSA, the relative water uptake for *sg-sub* is higher than for *WT* between 0 and 4 cm (+51.9% at 0–2 cm; +34.8% at 2–4 cm depth), and is lower than *WT* between 12 and 14 cm (−11.3%). For 60 days-old RSA, the relative water uptake for *sg-sub* is higher than *WT* between 0 and 6 cm (+203.2% at 0–2 cm; +123.1% at 2–4 cm; +63.9% at 4–6 cm), and is lower than *WT* between 12 and 22 cm (−33.3% at 12–14 cm; −36.7% at 14–16 cm; −30.2% at 16–18 cm; −17.2% at 18–20 cm; −8.1% at 20–22 cm) then higher again between 24 and 28 cm (+7.4% at 24–26 cm, +9.5% at 26–28 cm).

Altogether, these results show that, despite no apparent difference in whole root water uptake distribution, RSA without secondary growth (*sg-sub*) takes more water near the collar than the RSA with secondary growth but takes less water in deeper soil layers (Fig. 3C).

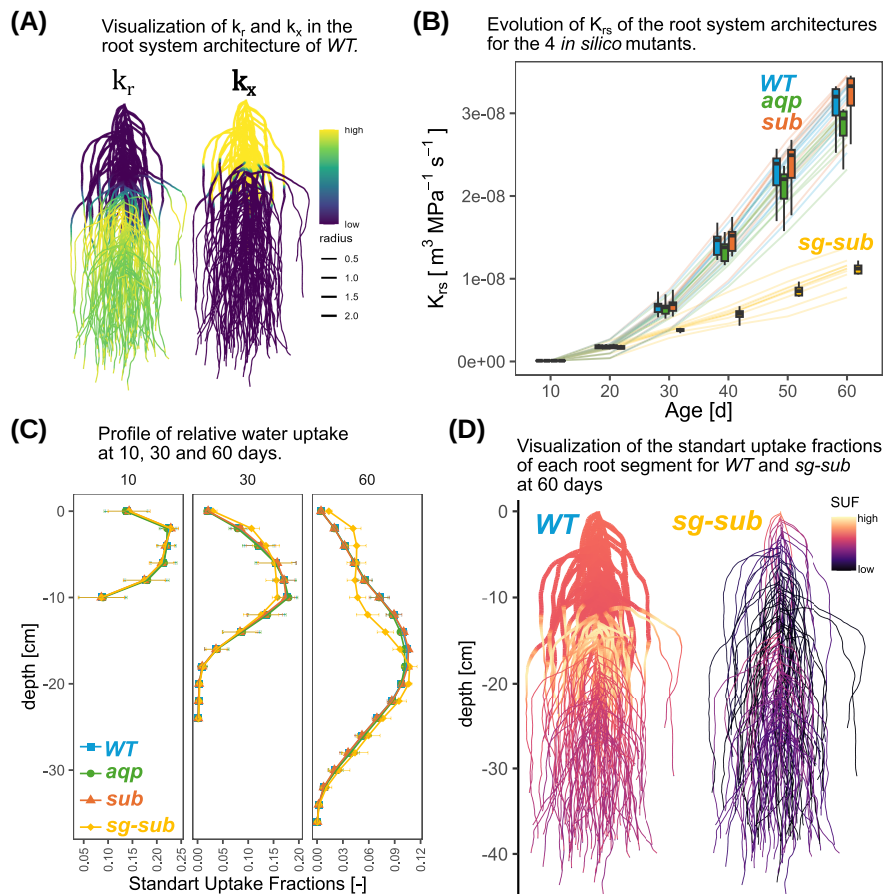
## 4. Discussion

### 4.1 Anatomy and secondary growth effects on hydraulic properties

Radial conductivity is fundamentally determined by anatomical traits, such as the number of cortex cells and the structure of the stele, explicitly modelled in frameworks like MECHA (Couvreur et al. 2018). Our results indicate that root age and radius account for a considerable proportion of the observed variability in radial hydraulic conductivity ( $k_r$ ). Both also show a good correlation with anatomical traits, justifying their use as proxies for predicting  $k_r$ . This aligns with observations reported by Heymans (2022).

With age, the number of stele parenchyma cells increases, as well as the number and the size of metaxylem vessels. The cambium generates secondary phloem and parenchyma cells, which increase the total size of the stele. The number of cortex cell layers decreases with time (from 3 to 4 in primary growth to 2 in secondary growth) and is not correlated with the increase of diameter (Fig. 2A). This reduction of cortex, at the opposite of what is observed in maize for example (see Chimungu et al. 2014, Heymans et al. 2020), contributes to an increase in  $k_r$  by reducing the length of the pathway for water to reach xylem vessels.

On the other hand, the number of cell layers of stele and phloem/cambium increases with age, especially in secondary growth zone. This increase makes them major contributors to the total increase in the root radius. With the increase of the stele comes an increase of xylem vessel number and cell size, which makes the specific axial hydraulic conductance  $k_x$  increase exponentially during formation of secondary growth (Fig. 2C). The increase of phloem and cambium layers



**Figure 3** Visualization and analysis of root system architectures and water uptake profiles in wild-type (WT) and in silico mutant plants. (A) Visualization of radial ( $k_r$ ) and axial ( $k_x$ ) hydraulic conductivities in the root system architecture of WT plants. The colour gradient is root radius (high to low). (B) Evolution of root system hydraulic conductivity ( $K_{rs}$ ) over time for the four in silico mutants. (C) Profile of relative water uptake at 10, 30 and 60 days. Standard uptake fractions are plotted against root depth for *sub*, WT, *aqp*, and *sg-sub* mutants, highlighting differences in water acquisition strategies over time. (D) Visualization of the standard uptake fractions of each root segment for WT and *sg-sub* in silico mutants.

eventually counters the effect of the thinner cortex layer and makes  $k_r$  drop (Fig. 2B).

## 4.2 Influence of apoplastic barriers deposition levels on root radial conductivity

### 4.2.1 Additional suberin does not reduce $k_r$ compared to lignin cap, in exodermis

Manzano et al. (2025) showed that in tomato, young parts of the roots present a lignin cap in the exodermis instead of Casparian strips. Cantó-Pastor et al. (2024) showed that tomato roots present suberization in exodermis but not in endodermis in 7 days old seedlings, which we also observed. We confirmed such observations through fluorescence microscopy, and we observed endodermal Casparian strip formation at the same timing.

Under the assumption that lignin is completely impermeable (Taiz and Zeiger 2002, Kang et al. 2019), our simulations suggest that an exodermal lignin cap strongly reduces  $k_r$  by 20% compared to only endodermal Casparian strips. In addition, when an impermeable lignin cap is present, the impact of additional exodermal suberin on  $k_r$  is overshadowed by the effect of the natural variability in root anatomies

(Fig. 2D). Our interpretation is that an impermeable lignin cap is sufficient to force water flow through the symplastic pathway, so adding an additional hydrophobic deposition on the opposite face of the exodermis has no substantial effect.

However, lignin being a highly heterogeneous polymer forming a network with other compounds of the cell wall, water permeability may still be possible due to porosity in the network (Deng et al. 2016, Couvreur et al. 2018). In such a case of permeable lignin cap, the tendency is reversed; there is no difference in  $k_r$  between permeable lignin cap in exodermis and no barrier in the exodermis, while there is a difference between permeable lignin cap and suberin (Fig. 2E). This highlights the importance of considering the permeability of apoplastic barriers in evaluating root hydraulic properties. It also motivates the need for experimental evaluation of such permeability.

It is worth to note that our approach does not include changes of other hydraulic parameters linked with hydrophobic deposition, like aquaporin expression (Hachez et al. 2006, Calvo-Polanco et al. 2021), which may play a significant role in regulating radial conductivity. Besides, our approach only considers the role of suberin as a barrier for the transmembrane and apoplastic transport of water, and does not consider

its other roles, like the preservation of homeostasis and nutrient transport, a response to various biotic/abiotic stresses, pathogen resistance or bacteria community composition (Andersen et al. 2015, Barberon et al. 2016, Salas-González et al. 2021, Liu and Kreszies 2023).

#### 4.2.2 $k_r$ is more sensitive to suberization in endodermis compared to exodermis

Our results show that in tomato, suberization would have a stronger impact on  $k_r$  if it were to happen in the endodermis rather than the exodermis (Fig. 2D). Adding a suberized endodermis makes  $k_r$  decrease by 46% compared to a simple endodermal Casparian strip, while adding an impermeable exodermal barrier (either lignin cap or suberin) to endodermal Casparian strip makes  $k_r$  decrease by 20% compared to only endodermal Casparian strips.

An explanation is that forcing a fully symplastic pathway across the endodermis is more of a bottleneck due to its smaller surface relative to the exodermis. In addition, in default MECHA inputs, exodermis cells present a higher plasmodesmata frequency compared to endodermis cells. These values were originally derived from maize (*Zea mays* L.), for which MECHA was initially parameterized (Clarkson et al. 1987). They were kept for tomato due to the absence of species-specific data. Given that the exodermis not only possesses a higher plasmodesmata frequency but also a greater surface area relative to the endodermis, the symplastic pathway is more conductive in the exodermis than in the endodermis. Consequently, suberization forcing a fully symplastic pathway provoked a more reduced  $k_r$  when happening in the endodermis than in the exodermis.

#### 4.3 Influence of aquaporins on root radial hydraulic conductivity

The contribution of aquaporins to membrane conductivity (kAQP) influences the root radial conductivity, depending on the deposition level (Table 4). The main hypothesis for that is that with no suberized layer, aquaporins facilitate the transmembrane flow of water, and the fraction of water reaching

the cytosol may then keep flowing radially from cell to cell through plasmodesmata, or back into the apoplast across membranes. Once the exodermis (or endodermis) is suberized, the only pathway across this cell layer requires transmembrane flow in the previous cell layer, followed by symplastic transport through plasmodesmata across the suberized cell layer. Due to their low conductances, low frequency and the absence of alternative pathway at this point, plasmodesmata then become the primary limit to radial water transport, and therefore reduce the sensitivity of radial conductivity to aquaporins (Gambetta et al. 2013, Heymans 2022).

#### 4.4 Application to root system hydraulic architectures: root system conductance and standard uptake fractions

Our results show that root system hydraulic conductance  $K_{rs}$  is highly influenced by secondary growth (Fig. 3B). The explanation is that secondary growth creates a much higher axial conductance in upper parts of the root (due to higher xylem vessels size and number), which enables the root system to transport more water and therefore take up more water from deeper soil layers. Indeed, the profile of the standard uptake fractions ( $SUF$ , which are the relative contribution of each segment to the total water uptake in homogenous soil) of the RSA without secondary growth shows higher relative water uptake near the collar, indicating that the root segments near the collar still take up water (Fig. 3C). But since the axial transport is limiting without secondary growth, one could expect that secondary growth root systems are able to take more water from deepest soil layers.

Another point is that, with secondary growth comes higher external surface of the root, which makes the radial conductance  $K_r$  increase, even if the radial conductivity  $k_r$  decreases. That means that secondary growth portions are still able to take up water radially, even with a much lower  $k_r$ . This is why the relative water uptake (represented by  $SUF$ ) does not decrease directly with the appearance of secondary growth. However, the relative water uptake ultimately collapses near

**Table 4** Summary of the linear regression of radial conductivity in function of hydrophobic barriers, aquaporin contribution, age and radius.

| Variable                        | Estimate | Std. error | T value | P value           |
|---------------------------------|----------|------------|---------|-------------------|
| (Intercept)                     | -2326    | 14.14      | -164.6  | <10 <sup>-4</sup> |
| Age                             | 15.33    | 0.2718     | 56.4    | <10 <sup>-4</sup> |
| Radius                          | -2307    | 24.54      | -94.01  | <10 <sup>-4</sup> |
| En.CS-Ex.LC                     | -264.5   | 18.7       | -14.14  | <10 <sup>-4</sup> |
| En.CS-Ex.Sub                    | -302     | 18.7       | -16.15  | <10 <sup>-4</sup> |
| En.Sub                          | -454.7   | 18.7       | -24.31  | <10 <sup>-4</sup> |
| En.Sub-Ex.CS                    | -581.5   | 18.7       | -31.09  | <10 <sup>-4</sup> |
| En.Sub-Ex.Sub                   | -701.3   | 18.7       | -37.5   | <10 <sup>-4</sup> |
| kAQP                            | 718 616  | 21 314     | 33.72   | <10 <sup>-4</sup> |
| En.CS-Ex.LC: kAQP               | 10 200   | 30 142     | 0.3384  | 0.7351            |
| En.CS-Ex.Sub: kAQP              | -33 898  | 30 142     | -1.125  | 0.2609            |
| En.Sub: kAQP                    | -409 658 | 30 142     | -13.59  | <10 <sup>-4</sup> |
| En.Sub-Ex.CS: kAQP              | -298 560 | 30 142     | -9.905  | <10 <sup>-4</sup> |
| En.Sub-Ex.Sub: kAQP             | -378 800 | 30 142     | -12.57  | <10 <sup>-4</sup> |
| Adjusted R <sup>2</sup> = 0.924 |          |            |         |                   |

the collar for two main reasons: there are less roots at this point in the profile, and the large axial conductance facilitates the delocalization of water uptake to other parts of the root system (Fig. 3C and D). This highlights the functional importance of the ratio between radial and axial conductivity (Draye et al. 2010).

#### 4.5 Opening to future works

In this work we developed a framework to couple plant hydraulic models from cells scale to root system scale, to integrate local knowledge into upscaled scale, similarly to what was developed in (Heymans 2022) but for dicotyledons. Our sensitivity analysis included aquaporins, exodermis apoplastic barriers and secondary growth.

All our hydraulic scenarios include a lignin cap in the exodermis, which appears in the first 2 cm of the root tip (Manzano et al. 2025). Our results show that that impermeable lignin cap has a strong effect on  $k_r$ , and therefore limits it from the very tip of the root. However, we showed that the permeability of lignin affects  $k_r$  and induce a first decrease of  $k_r$  before the deposition of suberin which then decrease it more effectively. Future simulations could include a more in-depth sensitivity analysis of lignin and suberin permeability and call for experimental measurements.

Our simulations also confirm that aquaporins highly influence transmembrane transport. However, we did not include in our sensitivity analysis the effect of plasmodesmata, which control symplastic water transport and could lead to significant changes in hydraulic properties. Unfortunately, data about plasmodesmata conductance and plasmodesmata frequencies in tomato is lacking. Further work could investigate the effect of plasmodesmata and include it into further sensitivity analysis.

More generally, the parameters of the hydraulic solver MECHA include ‘sub-cellular’ hydraulic properties like membrane permeability, cell wall conductivity, plasmodesmata frequency and conductance, and aquaporin contribution for specific tissues. These parameters were initially tuned for maize (Couvreur et al. 2018), and we conserved most of them for tomato. It is probably not accurate, since we expect these parameters to be species-dependant and to change with external factors. However, to our knowledge, there is a lack of data on these parameters for tomato, so we were unable to find new specific values. This is why we integrated a sensitivity analysis of the contribution of aquaporin to membrane permeability in our analysis. Although our parametrization of MECHA was kept simple here, it is worth noting that these sub-cellular parameters are expected to change with maturation and with apoplastic barrier deposition (Hachez et al. 2012, Gambetta et al. 2013). This would require an in-depth experimental analysis that has not yet, to our knowledge, been done in tomato. If such data happened to be available, our methodology would allow to easily run simulations on new sub-cellular properties values.

While time-intensive experimental measurements remain the most reliable way to estimate root hydraulic conductivities, our methodology only offers a potentially impactful trade-off between time and cost demanding measurements, and high-throughput integrated and systemic modelling. Our

framework enables quantitative assessment of root system hydraulic performance under varying environmental and physiological conditions. Its modular design supports the integration of novel hypotheses, such as comparative analyses of water-use efficiency in diverse root architectures (Chandrasekhar and Julkowska 2022, Koehler et al. 2025). Furthermore, the pipeline allows systematic evaluation of how alterations in hydraulic traits, whether structural (e.g. xylem anatomy, tissue composition) or functional (e.g. membrane permeability, apoplastic barriers) influence water acquisition, facilitating future studies on mutants with modified hydraulic properties (Cantó-Pastor et al. 2024, Manzano et al. 2025).

In this study, we have established a framework that integrates plant hydraulic models from the cellular level to the root system scale, enabling the incorporation of local insights into broader-scale analyses, akin to the approach done in (Heymans 2022) but tailored for dicotyledons. Our sensitivity analysis included factors such as aquaporins, exodermis, apoplastic barriers, and secondary growth. Future investigations using this framework could explore additional parameters, including plasmodesmata, and assess various combinations, such as different timings of lignin cap deposition.

Beyond the biological questions explored here, the framework itself may need to transition from R to Python for tighter integration with MECHA and improved interoperability within the modelling workflow. Integrating the framework into the OpenAlea ecosystem (Pradal et al. 2015) will further facilitate coupling with other functional-structural plant modelling tools and support the development of more comprehensive multiscale simulations. Another key improvement would be the realism of generated root anatomies by enabling finer control of cell dimensions and tissue organization, particularly to dicot root anatomy to better represent xylem element positioning and differentiation patterns. Ultimately, these developments should establish an open, extensible platform for linking developmental anatomy, tissue hydraulics, and root system function across scales.

## 5. Conclusion

In this work, we investigated the effect of the interplay between root developmental anatomy, secondary growth and hydrophobic deposition level on root hydraulic properties. We used and updated existing process-based computational models, and we integrated experimental root data and information on root system scale modelling.

Our results show that an impermeable exodermal lignin cap significantly reduces radial conductivity, but an additional layer of suberin in the exodermis does not when accounting for natural variability in root anatomies, thus questioning the role of suberin as hydrophobic barrier in exodermis. Our analysis also showed that lignin permeability influences radial conductivity.

Our simulations also show that secondary growth compensates for the decrease of conductivity by increasing the absorption surface, eventually making radial hydraulic conductance increase with the root age. Like so, secondary growth would compensate the effects of hydrophobic barriers in radial uptake, in addition to exponentially increase the axial

conductance, allowing for water from deepest root zones to move to the leaves. This is likely to be a critical feature in dicotyledons, where the totality of the water taken up by the root system must be funnelled through the root collar.

With our methodology, we confirmed experimental findings on the importance of exodermal hydrophobic depositions, and we created a modelling pipeline to simulate different scenarios of hydraulic properties, for example to investigate the hydrodynamics of apoplastic barriers mutants.

The models used in this study are purely process-based, user friendly and open source. The coupling and updates of such different multiscale functional structural plant models open avenues to improve and expand applications of digital avatars and studies on root system hydraulic dynamics.

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## Author contributions

Marco D'Agostino: writing—original draft, software (GRANAR), validation, formal analysis, investigation, data curation, visualization. Rémy Schoppach: software (GRANAR), formal analysis, validation. adrien heymans: methodology, software (GRANAR), writing—review & editing. Valentin Couvreur: methodology, software (MECHA), investigation, supervision, funding acquisition, writing—review & editing. Guillaume Lobet: conceptualization, supervision, project administration, funding acquisition, investigation, writing—review & editing.

## Supplementary material

Supplementary material is available at *in silico Plants* online.

## Conflicts of interest

Nothing to declare.

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## Data availability

The data and the codes underlying this article are available on Github at <https://github.com/MDagostino1/TRoot>, citable via Zenodo at <https://doi.org/10.5281/zenodo.18324981>, under Creative Common Attribution license. All cross-section pictures are available in Zenodo at <https://doi.org/10.5281/zenodo.20677865>.

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