

A Stroll Through the Developmental Landscape of Plants

Warre Dhondt¹, Michael Van de Voorde^{1,2}, and Prof. Dr. Ir. Steven Maere^{1,2}

¹Universiteit Gent

²Evolutionary Systems Biology, VIB-Ugent Center for Plant Systems Biology

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1 Introduction

During its development, a stem cell becomes gradually more restricted in form and function, at long last becoming one of a multitude of possible cell types that have been optimized during evolution to function as a part of a larger organism. It is this notion that C.H. Waddington tried to depict with his epigenetic landscape: that the sequence of developmental changes that a cell undergoes to achieve its final form proceeds along paths delineated by internal and external cues (Waddington, 2011; figure 1A). Waddington referred to such robust developmental paths as canalizations, but nowadays they are more commonly referred to as developmental trajectories (Waddington, 1957). This development is steered by molecular cues that depend on the cell's environment, among others. They determine the system's topology by creating additional possibilities via bifurcations, for example, or by taking away existing paths (Ferrell, 2012). This metaphorical epigenetic landscape has formed the ideological basis for much of our understanding of cellular developmental biology, and hypotheses often boil down to inquiries into said trajectories: what are the biological principles that steer the developmental trajectory of a cell?

Attempts at understanding the developmental trajectory at the level of organs, tissues, and more recently, single cells, have greatly benefited from advances in sequencing technologies and throughput (Goodwin et al., 2016). Within the plant community, the first organ-wide mappings of gene expression patterns date back to the early 2000s (Birnbaum et al., 2003; Brady et al., 2007). Transcriptome-wide expression profiling at the level of single cells using RNA-Seq was introduced in 2009, but at its inception, it could only profile a handful of cells (Tang et al., 2009). Key technological advancements have allowed for order-of-magnitude increases in throughput, with single-cell experiments currently routinely profiling thousands to hundreds of thousands of cells, making single-cell RNA-Seq (scRNA-Seq) a mainstay in modern biological research (Svensson et al., 2018). These advancements did not go unnoticed in plant developmental biology, where single-cell resolution gene expression *atlases* of the *Arabidopsis thaliana* root (Denyer et al., 2019; Shahan et al., 2022; Wendrich et al., 2020), shoot (Zhang et al., 2021), vascular tissue (Kim et al., 2021; Otero et al., 2022) and seed (Picard et al., 2021) have been published in the last 4 years, to name a few.

Such single-cell expression¹ experiments offer a wealth of information on dynamic processes such as decision making during differentiation, and, in some cases, can be considered a direct readout of Waddington's developmental landscape (Griffiths et al., 2018; Trapnell, 2015). This has triggered a surge in computational methods, classified under the title *Trajectory Inference* (TI), that aim to infer the dynamic processes contained in the static snapshot that a single-cell experiment has to offer. Trajectory inference algorithms aim to reconstruct the developmental trajectory from a starting cell to another cell by assuming that cells that lie on the path between the starting and terminal cells represent

¹Other common single-cell 'omics modalities include chromatin availability, DNA methylation, genomics and even proteomics (Vandereyken et al., 2023)

developmental intermediates, and then, sometimes quite literally, connecting the dots (Bergen et al., 2020; Haghverdi et al., 2016; Street et al., 2018; Trapnell et al., 2014; figure 1B). Trajectory inference methods differ in their assumptions, their robustness, the underlying algorithm, or the topologies they can detect; the performance of most popular TI methods has been thoroughly reviewed in Saelens et al., 2019. Trajectory inference algorithms assign cells along the trajectory a *pseudotime*, a unitless measure whose purpose is to give an indication of where along the trajectory the cell is located. Because of this pseudotime assignment, a lineage and its cells can be treated as a pseudo-time-series experiment, and one can identify temporal expression patterns or differential gene expression within and between lineages (Van den Berge et al., 2020), dynamic gene regulatory networks (Nguyen et al., 2020), or compare trajectories (Alpert et al., 2018; Deconinck et al., 2021; figure 1C).

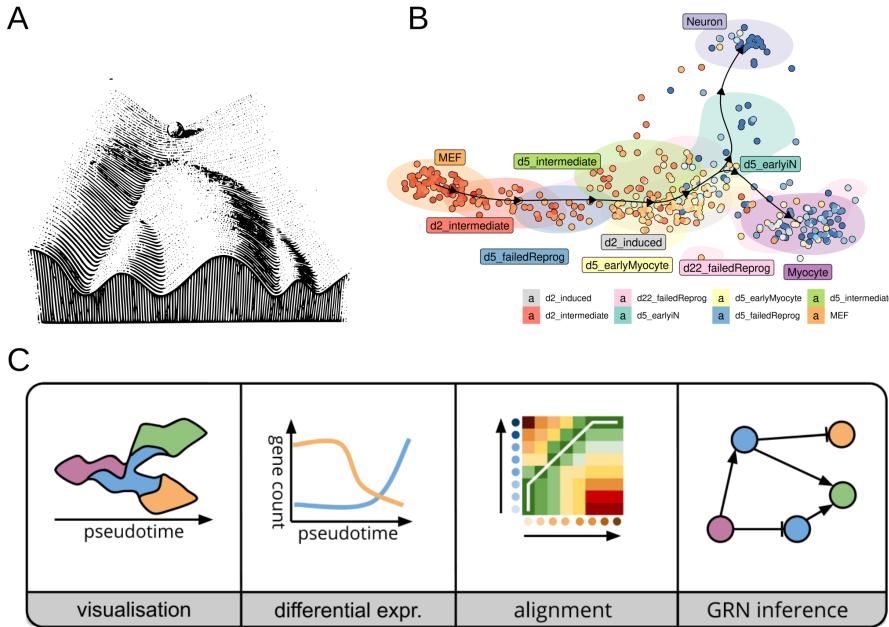


Figure 1: An overview of trajectory inference and possible downstream analyses.

(A) Waddington's epigenetic landscape. Illustration from (Waddington, 1957). (B) Trajectory inference on the bifurcating developmental trajectory of mouse embryonic fibroblasts (Robrecht Cannoodt, n.d.). (C) Possible analyses downstream of trajectory inference include visualization, trajectory-based differential expression, alignment of trajectories and inference of gene regulatory networks. Figure adapted from Deconinck et al., 2021.

Despite trajectory inference being developed by the single-cell genomics field, TI's conceptual framework can be applied to other applications where one wishes to study the temporal dynamics of an observable based on high-dimensional data. For example, the Maere lab (Center for Plant Systems Biology, VIB-UGent) aims to use TI methods on single plants to try and decipher developmental processes at the level of plant field trials. The plant root tip is one model system where TI methods have been extensively used in recent years (Denyer et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Wang et al., 2020; Zhang et al., 2021; Zhang et al., 2019). Stem cells from the root apical meristem are committed to a cell type according to their position along the radial axis, giving the root a concentric organization of its tissues (*Maybe include figure?*). Meristematic cells from the root apical meristem divide continuously to provide new cells to developing root tissue. As the root elongates, the devel-

oping cells stray away further from the stem cell niche and start to lose their proliferative status, while they increase in length and gain their cellular identity in the elongation and maturation zones (Petricka et al., 2012). This defined radial pattern of cell specification and longitudinal differentiation axis have been studied thoroughly and make the root tip a good model system for computationally inferring developmental lineages using TI (Seyfferth et al., 2021). The pathways underlying the differentiation of hair cells (trichoblasts) and non-hair cells (atrichoblasts) in the *Arabidopsis* epidermis have been characterized in quite some detail. Epidermal cell patterning is the result of a complex interplay between cell-cell communication involving cortical and epidermal cells, the exchange of mobile transcription factors, and lateral inhibition (Balcerowicz et al., 2015; Bruex et al., 2012; Salazar-Henao et al., 2016; Schiefelbein et al., 2014). This research project illustrates the use cases of trajectory inference on a scRNA-Seq dataset of the *Arabidopsis* root tip from Denyer et al., 2019. We show how trajectory inference can be used to asses pseudo-temporal dynamics of gene expression of known regulators of the atrichoblast-trichoblast differentiation axis, and how the information gained from TI can be applied to elucidate the regulation of epidermal cell patterning.

2 Aim

Recent advances in sequencing technologies have established single-cell RNA-Seq as a mainstay in modern biological research. This is illustrated by the surge of published single-cell expression atlases, including multiple of the *Arabidopsis thaliana* root. The first aim of this project is to familiarize oneself with current community standards for processing scRNA-Seq data (see Amezquita et al., 2019; Luecken and Theis, 2019), exploring different options along the way to find out their advantages and disadvantages. This includes, but is not limited to, exploratory data analysis and quality control, clustering methods such as Louvain community detection, dimensionality reduction (including t-distributed stochastic neighbor embedding and Uniform Manifold Approximation and Projection) and cell type annotation. The dataset of choice is a publicly available scRNA-Seq dataset of the *Arabidopsis* root from Denyer et al., 2019, who mainly focused on analyzing dynamic gene expression within differentiating trichoblasts, a type of epidermal cell. The analysis presented in the original publication only scratched the surface of epidermal cell patterning, which is the result of the coordination between multiple developmental processes such as cell-cell communication, lateral inhibition, and feedback loops. The second aim of this project is therefore to try and reproduce the findings of Denyer et al., 2019 (to a certain extent) and to explore epidermal cell patterning using computational techniques that leverage the dynamic nature of the data. This reanalysis starts off with trajectory inference to identify developmental lineages using Slingshot (Street et al., 2018). Once they have been identified, the reconstructed trajectories can be treated as a pseudo-time-series experiment, that allows us to assess biological patterns in a temporal manner to gain insight into epidermal cell differentiation. TradeSeq (Van den Berge et al., 2020) offers a statistical framework to compare pseudo-temporal patterns of gene expression both within a single trajectory, or between developmental trajectories. Within the confines of this project, TradeSeq can be used to analyze the dynamic expression of known regulators of epidermal cell patterning and compare how the expression patterns of these regulators may vary between developing cell types. Gene ontology enrichment can be an asset here to extract biological meaning from the (likely) large number of genes in some of the analyses (Ashburner et al., 2000). If time permits, dynamical regulatory network inference could be used as a more rigorous means of detecting regulatory modules whose activity changes along the developmental axes. Further, computational approaches that try to determine cell-cell communication based on single-cell expression data (Efremova et al., 2020; Xu et al., 2022) can be used to add interaction between cell types as an additional piece in the biological puzzle.

3 Overview of Techniques

Exploratory data analysis and preprocessing in Seurat

- Quality control of scRNA-Seq libraries
- Normalization methods (log-normalization, variance-stabilizing normalizations)
- Louvain clustering
- Dimensionality reduction (PCA, t-SNE, UMAP, Diffusion maps, PaCMAP)
- Cluster and cell type annotation (cell-type markers, correlation-based)

Trajectory inference and differential expression

- Reconstructing trajectories using Slingshot
- Pseudotime differential expression analysis using TradeSeq

Additional analyses

- GO functional enrichment analysis using GOseq
- Inference of ligand-receptor interactions using PlantPhoneDB

General tools

- Various visualization methods (ComplexHeatmap package, ggplot)
- Coding in R
- Using a computer cluster
- Version control (Git)

4 Materials and Methods

4.1 Preprocessing, Clustering, Dimensionality reduction

The gene by cell expression matrix with raw scRNA-Seq read counts for the wild-type *Arabidopsis thaliana* root atlas from Denyer et al., 2019 were retrieved from the Gene Expression Omnibus database under accession [GSE123818](#). All downstream processing was performed using the Seurat V4 framework (Hao et al., 2021). Genes that are known to be differentially expressed upon protoplasting (\log_2 fold change >2 , $p < 0.05$) were dismissed prior to further analysis as in Denyer et al., 2019. Initially, putative high quality cells were identified by filtering the raw library for cells with $<5\%$ of genes mapping to mitochondrial and chloroplast genes, as well as ad hoc cutoffs for library size and number of counts. However, mapping the filtered cells to the final processed and annotated dataset via mutual nearest neighbors showed that cells that were filtered belonged predominantly to the one cell type, and this led to removing approximately 30% of all trichoblasts (χ^2 -test, $p = 2 \times 10^{-4}$). The cells were therefore not filtered based on the number of counts and/or features. The counts were normalized using the variance stabilizing regularized negative binomial regression as implemented in `SCTransform`, using the % reads mapping to mitochondrial and chloroplast genes as additional regressors (Hafemeister and Satija, 2019). The 3000 most variable genes were identified as those having the highest residual variance in this regression model. The dimensionality of the data was reduced by performing principal components analysis on the top 3000 most variable genes. Graph-based clustering was performed using the Louvain algorithm on the first 30 principal components (Seurat `FindClusters`, resolution = 1). Two-dimensional embeddings were generated using the first 30 principal components as input for the Seurat function `RunUMAP` using standard settings (`n.neighbors = 30`; `min.dist = 0.3`; 500 epochs).

4.2 Annotation of Clusters and Cell Types

For each cluster, markers were identified using Wilcoxon Rank Sum tests. The cluster markers were filtered for an average \log_2 fold change >0.75 and to be expressed in $>25\%$ of cells of that cluster. Only markers with a positive log fold-change for a given cluster compared to the remaining cells were considered. First, these markers were mapped to the cell-type specific markers from Shahan et al., 2022 to infer the cell type associated with the clusters. Second, for every cluster, the top 30 differentially expressed markers ranked by fold change were mapped to tissue-specific cell type markers from Brady et al., 2007. These cluster annotations were reconciled with expression patterns of known developmental genes and manual inspection of the cluster-specific markers for the final cell-type annotation. Further, individual cells were annotated by their ploidy levels (2C, 4C, 8C, 16C) and developmental zone (meristem, elongation, maturation) by calculating the Pearson correlation coefficient with bulk RNA-seq reference expression profiles (Bhosale et al., 2018; Brady et al., 2007), using the annotation process kindly provided in the Github repository of Shahan et al., 2022.

4.3 Trajectory inference and Trajectory Differential Expression

For trajectory analysis, Slingshot (Street et al., 2018) was used to build a minimum-spanning tree on the a subset of clusters 3, 4, 5, 6, 9, 10, 11, 14 an 18, fixing trichoblast (cluster 9), atrichoblast (cluster 6) and cortex (cluster 14) as terminal clusters and cluster 10 (QC/meristematic cells) as starting cluster. Simultaneous principal curves were then fitted based on this minimum spanning to infer smooth trajectories, and pseudotimes were assigned to each cell based on these principal curves. Next, for every lineage, a negative binomial generalized additive model was fitted on the global top 3000 variable genes using TradeSeq (Van den Berge et al., 2020). The generalized additive models were fitted with six knots, which was determined to be optimal using the akaike information criterion in `evaluateK`. Genes that were differentially expressed between differentiated trichoblasts and atrichoblasts were detected using the tradeSeq `diffEndTest`.

4.4 Pseudotime Expression Heatmaps

First, genes that are differentially expressed across pseudotime were identified using the TradeSeq `associationTest`, retaining only those genes whose Wald test statistic had a $p < 0.01$. For those genes, the mean smoother was predicted along 30 pseudotime bins in each lineage with `predictSmooth`. Per lineage, the genes were grouped into 7 clusters via hierarchical clustering using Ward's linkage. For each cluster, over-represented Gene Ontology categories were identified using GOseq (Young et al., 2010). For all genes in the dataset, GO annotations ('biological process') were retrieved from plants.ensembl.org using BioMart. To account for the gene selection bias based on its length, a probability weighting function was estimated from the raw count matrix. Over-represented categories were identified as those having a Fisher's exact test (GoSeq `'Hypergeometric'` method) $p < 0.05$ after Benjamini-Hochberg (BH) correction. The heatmaps were produced using the ComplexHeatmap package in R (Gu et al., 2016). For the heatmap annotations, each bin's developmental zone and ploidy level (see above for details on annotation) was determined by majority rule within that bin.

4.5 Ligand-Receptor Interactions

For trichoblast, atichoblast and cortex cells, ligand-receptor (LR) interactions were inferred from co-expression data using PlantPhoneDB (Xu et al., 2022). For all cell type combinations, LR interactions were scored using the mean ligand and receptor expression levels as in Efremova et al., 2020, and significant scores were identified using permutation tests (100 iterations). For significant LR pairs (BH-corrected $p < 0.01$), their score across 30 pseudotime bins was determined as the mean of the TradeSeq smoother of the ligand in receptor in the respective cell types from which the interaction was inferred.

5 Results

5.1 Single-cell transcriptomics of the *Arabidopsis* root

The analysis began with processing the scRNA-Seq dataset of the *Arabidopsis thaliana* root (Denyer et al., 2019). Cells that are similar in terms of gene expression were clustered into 19 groups, and these clusters were annotated by mapping cluster-specific gene expression onto marker genes of isolated *Arabidopsis* root tissues from Brady et al., 2007 (figure 2A-C). The UMAP shows a disconnected, star-like structure consisting of the central meristematic clusters extending out to different developmental lineages. Central clusters 3, 4, 10 and 16 all mapped to meristematic xylem tissue, but closer inspection of the identified marker genes showed that these were predominantly markers for cells with high mitotic/proliferative activity, without markers related to xylem development. For example, clusters 3, 4, and 16 highly express genes related to DNA replication or nucleolar functionality, including genes coding for histone family proteins and ribosomal proteins such as RPL16A (AT2G42740) (Bernstein and Baserga, 2004; Bernstein et al., 2007). Further, the mitotic regulators cyclin CYCB1;1 and Aurora kinase AUR1 are highly expressed in cluster 16, both of which are commonly used as markers for actively dividing cells (Schnittger and Veylder, 2018; Weimer et al., 2016). Cluster 10 additionally expresses QC marker GOLVEN 6/RGF8 (Fernandez et al., 2013), and the gene for ent-kaurene oxidase 1 (GA3, AT5G25900), which is involved in gibberellin biosynthesis, a process that has been associated with QC identity (Nawy et al., 2005). Cluster 10 therefore likely consists of QC cells as well as proliferating cells.

The central mass of meristematic cells radiates outwards towards 5 distinct lineages. These lineages were identified as vascular cells (clusters 8, 12, 13), cortex and endodermis (14, 18, and 15, respectively), trichoblast and atrichoblast (5, 9 and 6, 11, resp.) and the root cap, comprising cells of the central columella (0, 17) and lateral root cap (1, 2). Many of the genes that regulate root cap development are differentially expressed upon protoplasting and were therefore not included in the analysis. This is reflected in the comparatively low agreement on the identify of root cap cell types compared to other cell types. Interestingly, cells that are located terminally in the root-cap lineage highly express genes involved in peroxisome biogenesis (PEROXIN family members), glucosinolate metabolism, and biogenesis of ER bodies, which are organelles that are involved in stress responses and immunity (Sarkar et al., 2020; Su et al., 2019). Other cluster-specific marker genes are involved responses against abiotic stress, such as those encoding defensin-like proteins (*TI1*, AT2G43510), GENOMES UNCOUPLED 2² (*GUN2*, AT2G26670), SENESCENCE 1 (*SEN1*, AT4G35770) and WRKY26 (AT5G07100; Phukan et al., 2016). Such stress-specific gene expression could play into the root cap's protective role against biotic and abiotic stresses (Kumar and Iyer-Pascuzzi, 2020), or, alternatively, this stress-response could be the result of programmed cell death during root cap cell sloughing (Kumpf and Nowack, 2015). This hypothesis could not be tested, as the genes involved in root cap cell sloughing are differentially expressed upon protoplasting and were

²*GUN2* is involved in stress-induced chloroplast dysfunction (Crawford et al., 2017)

therefore not included in the analysis. Further, the resolution between the different vascular cell types is comparatively low. This lack of resolution is a known problem in plant single-cell transcriptomics and has been attributed to a high overlap in gene expression between vascular cells and its associated pericycle (Parizot et al., 2012), as well as issues with accessibility, causing to vascular cells to be underrepresented in single-cell atlases (Otero et al., 2022). Lastly, the trichoblast and atrichoblast epidermal cells were identified by the characteristic expression of *GLABRA 2* (*GL2*, AT1G79840), a repressor of the trichoblast fate in atrichoblast cells, and the root hair tip growth regulator *COBRA-LIKE 9* (*COBL9*, AT5G49270), respectively (Jones et al., 2005; Masucci et al., 1996).

As the root elongates, developing cells stray away further from the stem cell niche and start to lose their proliferative status, while they increase in length and gain their cellular identity in the elongation and maturation zones (Petricka et al., 2012). During this differentiation, plant cells trade the standard, mitotic cell cycle for endoreduplication and the concomitant increase in cell endoploidy. To infer the developmental status of the cells in our dataset, cellular gene expression profiles were correlated with bulk RNA-Seq reference expression profiles for different root cell ploidy levels and developmental zones (Shahan et al., 2022; figure 2D-E). In agreement with the mutually exclusive nature of the mitotic cell cycle and endoreduplication, meristematic cells were annotated as having regular ploidy (2C), or having undergone a single endocycle (4C). The transition from the central meristematic cells to the different developing lineages is accompanied by an increase in ploidy to 8C. The highest ploidy level occurs predominantly in trichoblast cells nearing the maturation stage, with other 16C cells scattered across other mature cell types in small numbers. This additional endoreduplication in root hair cells is a known phenomenon, but the biological reasons for this are as yet unknown (Bhosale et al., 2018). The transition between the developmental stages is delayed compared to the increase in ploidy levels. This is in agreement with Hayashi et al., 2013, and implies that an increase in cellular ploidy precedes the transition between developmental zones of the root. Importantly, the pattern of the endoploidy and developmental stage annotations confirm that the developmental lineages stretch from the central meristematic cells outward to the differentiated cell types, and that the different lineages encompass the entire development from immature cells in the root apical meristem to mature cell types.

5.2 Developmental Trajectories of Root Epidermal Cells

Next, we set out to determine patterns of gene expression that are involved in epidermal cell differentiation. The developmental lineages for trichoblast, atrichoblast, and cortex cells were reconstructed using Slingshot (Street et al., 2018), starting from cluster 10 (QC and meristematic cells), and ending in the differentiated cell types. This approach is supported by the patterns observed in the ploidy and developmental stage annotations, and ensures a pseudotime ordering of the cells that represents a biologically meaningful signal (figure 3A). The epidermal and cortex trajectories diverge early on during the meristematic stage, which is in agreement with the cell types developing from distinct types of initial cells (the cortex-endodermis and epidermal initials, respectively). The trichoblast and atri-

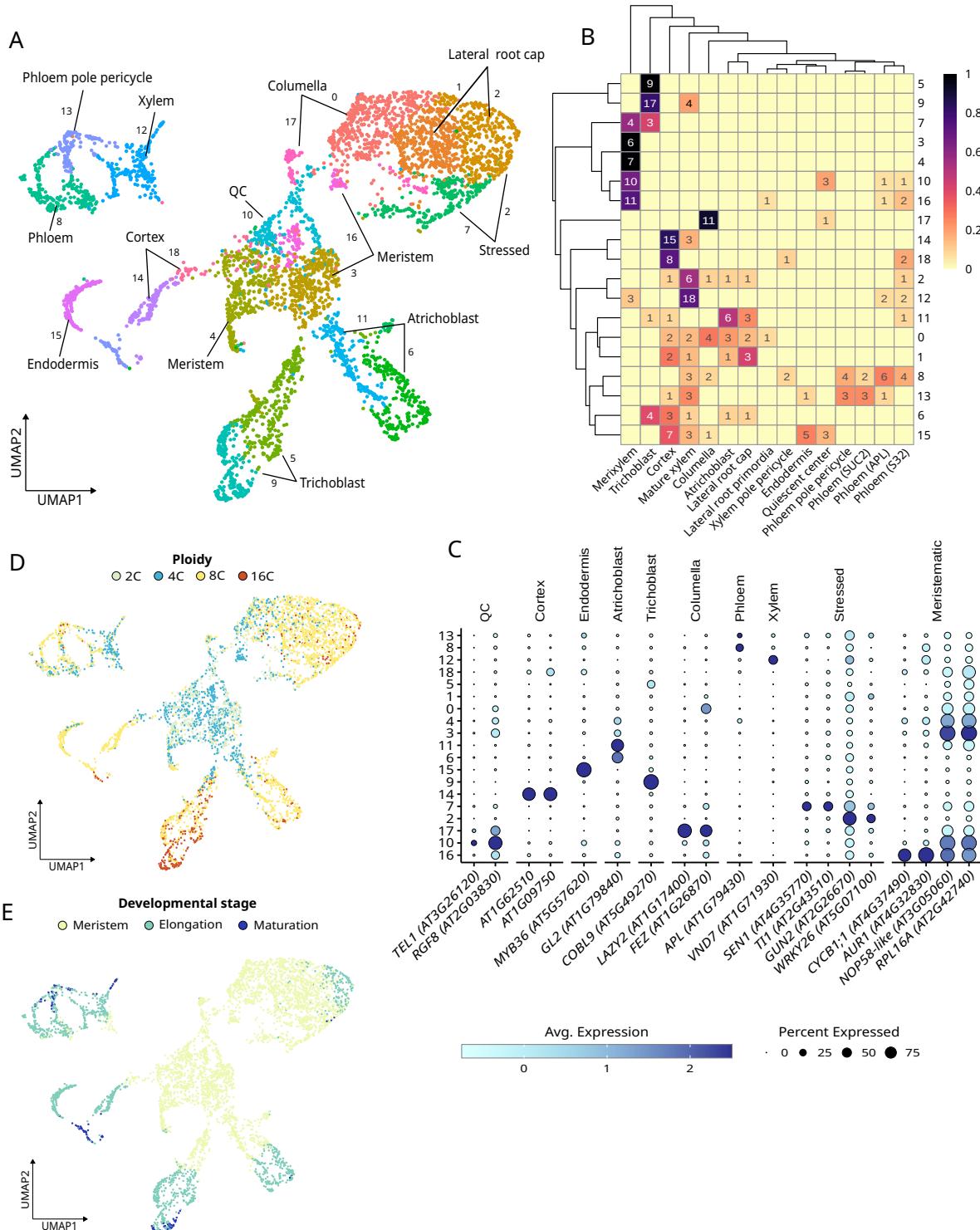


Figure 2: Cell types of the *Arabidopsis* Root Identified by scRNA-Seq.

(A) UMAP of the 4727 *Arabidopsis* root cells. The (sub)clusters are colored and named according to their cell type annotation. Numbers besides the arrows indicate the cluster numbering as referred to in the text. (B) Heatmap showing the contribution of marker genes of different cell types to the cluster identity. The top 50 differentially expressed genes per cluster were mapped to marker genes of bulk RNA-Seq data of plant root tissue sections. The numbers in the matrix represent the number of DE genes of the top 50 of that cluster mapping to a certain tissue. The row colors are the normalized contribution of the cell types to the top DE genes of a given cluster. (C) Dot plot showing expression of known cell type marker genes per cell cluster identified in the scRNA-Seq dataset. Dot sizes represent % expression in a cluster, color reflects average normalized and scaled expression. (D-E) UMAP with cells annotated according to their inferred ploidy level (D) and developmental zone (E). The ploidy level and developmental zone of each cell was identified by correlation with bulk RNA-seq reference expression profiles.

choblasts trajectories have a larger part of their trajectory in common and emerge from a shared body of meristematic cells (cluster 3).

To determine the temporal patterns of gene expression within these lineages, the most variable genes that showed significant differential expression across pseudotime were grouped based on their expression across 30 pseudotime bins, and the resulting clusters were evaluated for functional enrichment of GO biological processes³. This resulted in the identification of gene modules with distinct expression patterns that are functionally tailored towards the developmental stage and cell type (figure 3B). The early meristematic stage is functionally enriched for genes involved in transcriptional activity and genome architecture, as well as auxin-activated and regulating processes. The latter is surprise, given auxin's cardinal role in pattern specification in the root stem cell niche (Pardal and Heidstra, 2021). By the time ploidy levels increase towards the later meristematic stage, these is a surge gene expression for ribosome function and translation, which could be required to maintain steady-state protein concentrations for endoreduplication-driven cell growth. The developmental transition from the meristematic to the elongation stage is accompanied by an increase gene expression for cell wall biogenesis and remodelling. Elongation-stage trichoblasts are also functionally enriched for synthesis of the thalianol, a triterpenene phytohormone. Thalianol biosynthesis is regulated by three genes grouped in an operon-like fashion. Thalianol is a relatively unexplored phytohormone, but the genes involved in its biosynthesis are known to be expressed predominantly in epidermal cells towards elongation/mature developmental stages (Field et al., 2011), and the hormone is a modulator of root growth (Bai et al., 2021). Late-stage gene expression in either cell type is largely targeted towards transmembrane transport and stress responses. At this point, trichoblasts additionally express genes for cell wall remodelling and tip growth, and known regulators of root hair development, including *ROP4*, *ROOT HAIR SPECIFIC 11* and *13*, and *ROOT HAIR DEFECTIVE 6-LIKE 2* and *4* (figure 3C). The increasing expression of the latter, *RSL4*, harmonizes with the decreasing expression of *GLABRA 2*. *GLABRA 2* is master regulator in atrichoblast specification and inhibits a set of bHLH transcription factors that activate a cascade of root hair cell-specifying genes in developing trichoblasts (Lin et al., 2015). The dynamic expression patterns of *RSL4* and *GL2* observed here support the findings of Qiu et al., 2021, who hypothesized the involvement of *RSL4* in the inhibition of *GL2*-mediated atrichoblast specification in trichoblast cells (figure 3C, bottom left).

5.3 The role of ligand-receptor interactions in trichoblast development

Trichoblasts and atrichoblasts patterning in the root epidermis occurs in a position-dependent manner (Balcerowicz et al., 2015). Intercellular signals produced by cells of the neighboring cortex specify epidermal cells to become either hair cells (H-cells) or non-hair cells (N-cells). First, putative ligand-receptor (LR) interactions were inferred based on their co-expression between the different clusters. Next, the mean expression scores of significant interactions was determined across 30 pseudotime

³See methods for details

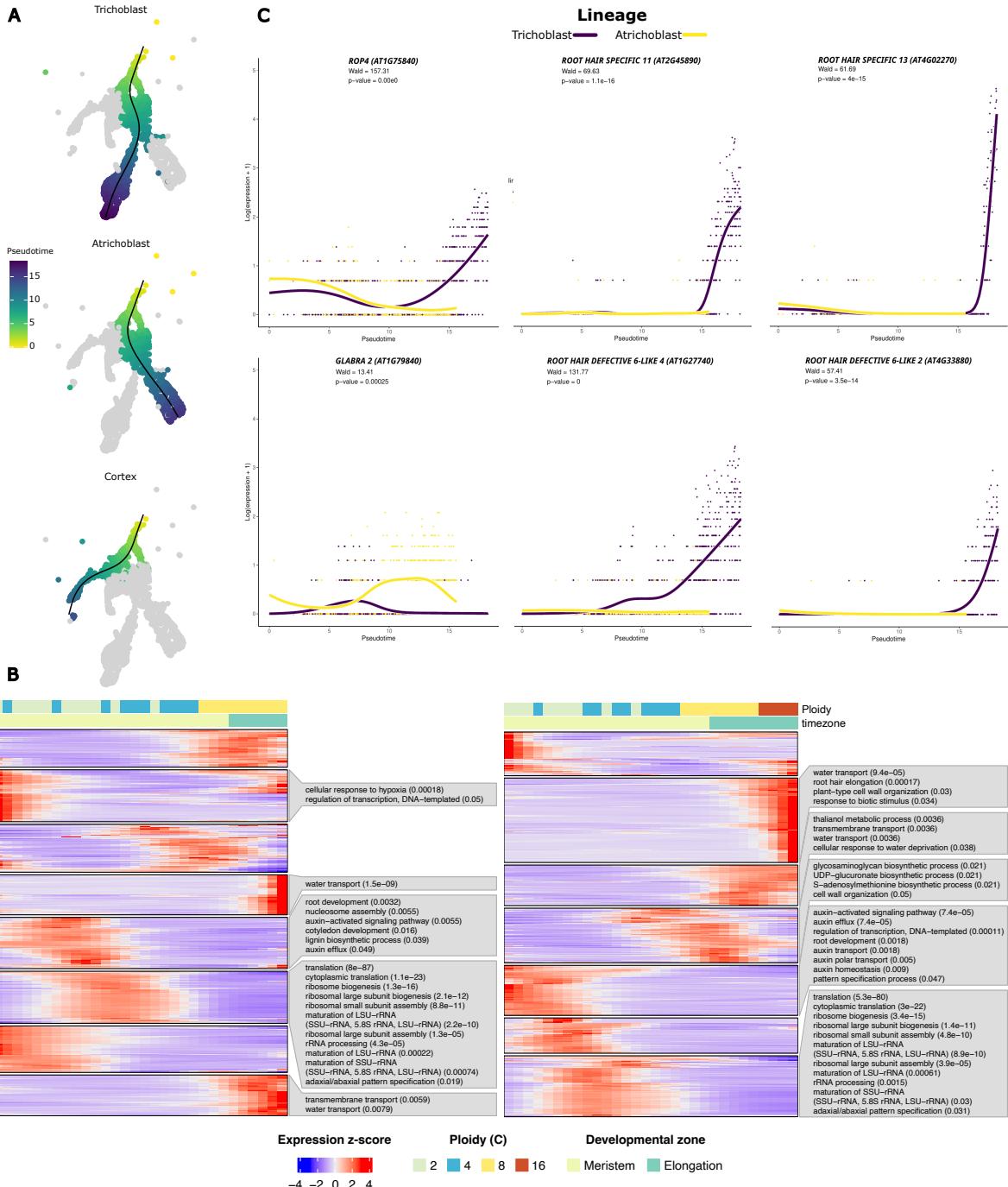


Figure 3: Differential expression of root hair growth regulators across pseudotime. (A) UMAP describing the developmental lineages from a central cluster (QC/meristematic cells) to differentiated trichoblast, atrichoblast and cortex cell types. The lineages were identified via Slingshot; the black line indicates the principal curve passing through the cells belonging to that lineage. (B) For the atrichoblast (left) and trichoblast (right) lineages, the 3000 most variable genes were clustered according to their expression across 30 pseudotime bins. Each cluster was tested for functional enrichment in GO biological processes using the hypergeometric test (BH-corrected p shown between brackets). The developmental zone and ploidy annotations on top correspond were determined by majority-rule in each pseudotime bin.

bins to assess the dynamics of these L-R interactions, and LR pairs were clustered according to their expression across pseudotime (figure). Computational tools that try to infer cell-cell communication from co-expression data typically consider all pairwise combination of cell types. Because a cell's developmental state depends on its position along the longitudinal axis of the root, we imposed an additional requirement that cells cannot interact with less mature cells from the same developmental lineage. The majority of significant LR pairs are most highly expressed in the later developmental stages (figure panel A). For each cluster of LR-pairs with similar temporal dynamics, the top scoring ligand-receptor pairs are dominated by interactions involving highly abundant cell wall proteins, such as those of the arabinogalactan protein family (AGP). The specificity of the proposed interactions is readily apparent when looking at the expression of the involved genes across pseudotime (figure panel B), and makes a good case for developing a method to inferring CCC along developmental trajectories that takes into account the issues stated above.

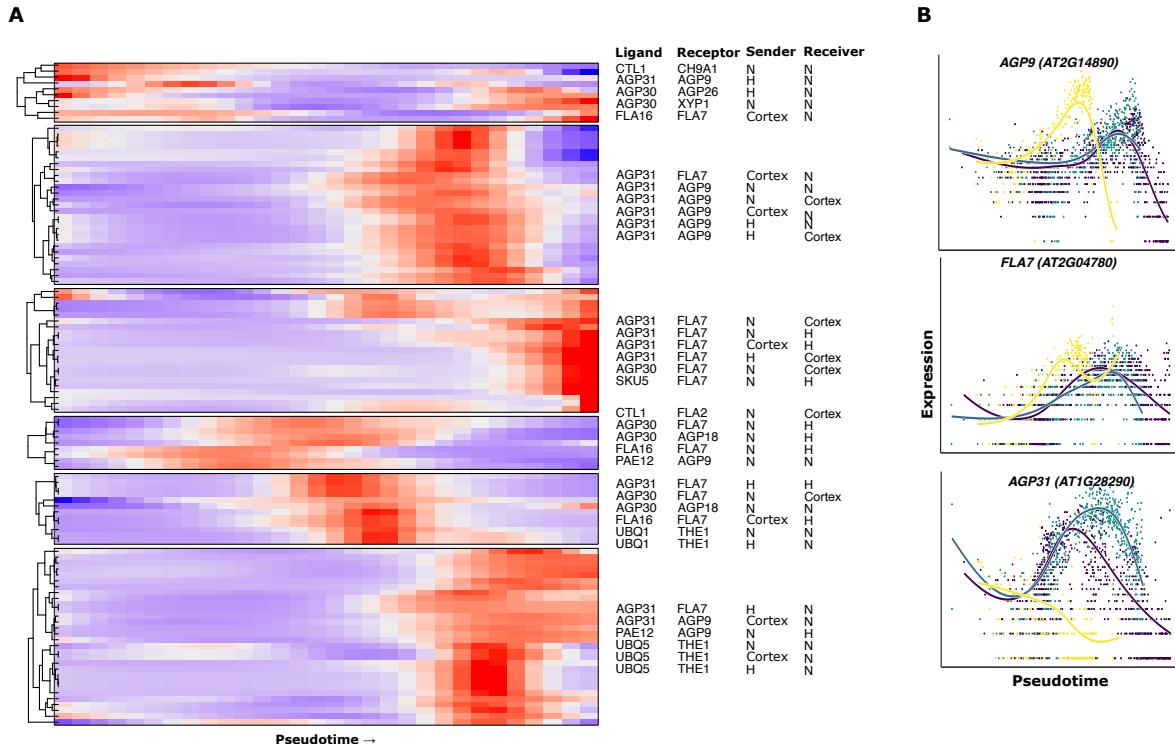


Figure 4: Crosstalk between cells of the developing root inferred from single-cell expression data. Add description!

6 Discussion

In this project we reanalyzed a single-cell RNA-Seq dataset of the *Arabidopsis* root from Denyer et al., 2019 with the goal of illustrating how trajectory inference can be used to study epidermal cell differentiation. In previous work, trichoblast development was studied by looking at dynamic enrichment of transcription factor motifs (Jean-Baptiste et al., 2019) or functional enrichment and gene regulatory

networks (Denyer et al., 2019). This project expanded on the latter approach by comparing the temporal dynamics of functional specialization between trichoblasts and their atrichoblast counterpart, and layering information about the cell's ploidy and developmental zone on top of this. We reconstructed developmental trajectories for trichoblast and atrichoblast differentiation, and identified temporally regulated gene modules whose functional enrichment caters towards the requirements of the cell's developmental stage. In agreement with established work (Balcerowicz et al., 2015), trichoblasts only start to functionally diverge from atrichoblasts starting from the elongation zone, whereas a transcriptomic and morphological can be detected much earlier, in the meristematic zone.

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