A Stroll Through the Developmental Landscape of Plants

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1 Results

1.1 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 *Arabdopsis* root tissues from Brady et al., 2007 (figure 1A-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 in involved responses against abiotic stress, such as those encoding defensin-like proteins (*TI1, AT2G43510*), GENOMES UNCOUPLED 2[1] (*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

[1] 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 1D-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 not known (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.

1.2 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 2A). 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-

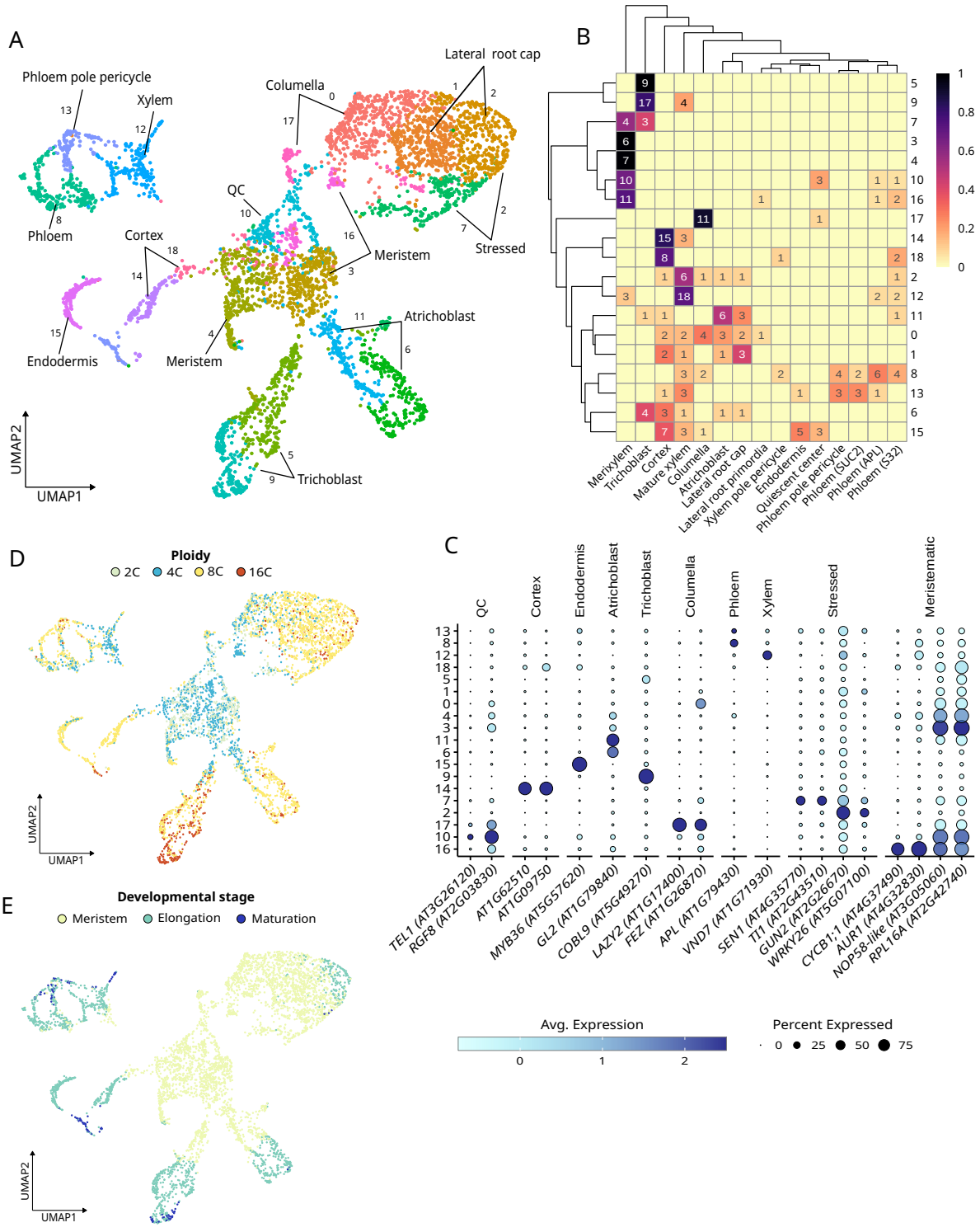

Figure 1: Cell types of the *Arabidopsis* Root Identified by scRNA-Seq.
(A) UMAP of the 4727 *Arabidopsis* root cells. The (sub)clusters are colored and named according to their cell type annotation. Numbers besides the arrows indicate the cluster numbering as referred to in the text. (B) Heatmap showing 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 processes[2]. This resulted in the identification of gene modules with distinct expression patterns that are functionally tailored towards the developmental stage and cell type (figure 2B). 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 triterpentene 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 2C). Recent findings of Qiu et al., 2021 implied mutual inhibition between ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4) and GLABRA 2, a transcription factor. 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 involvement of RSL4 in the inhibition of GL2, be it direct or indirect, is in agreement with our findings on the temporal dynamics of *RSL4* and *GL2* expression within the trichoblast lineage, where the decrease in *GL2* harmonizes with the increase in *RSL4* (figure 2C, bottom left).

[2] See methods for details

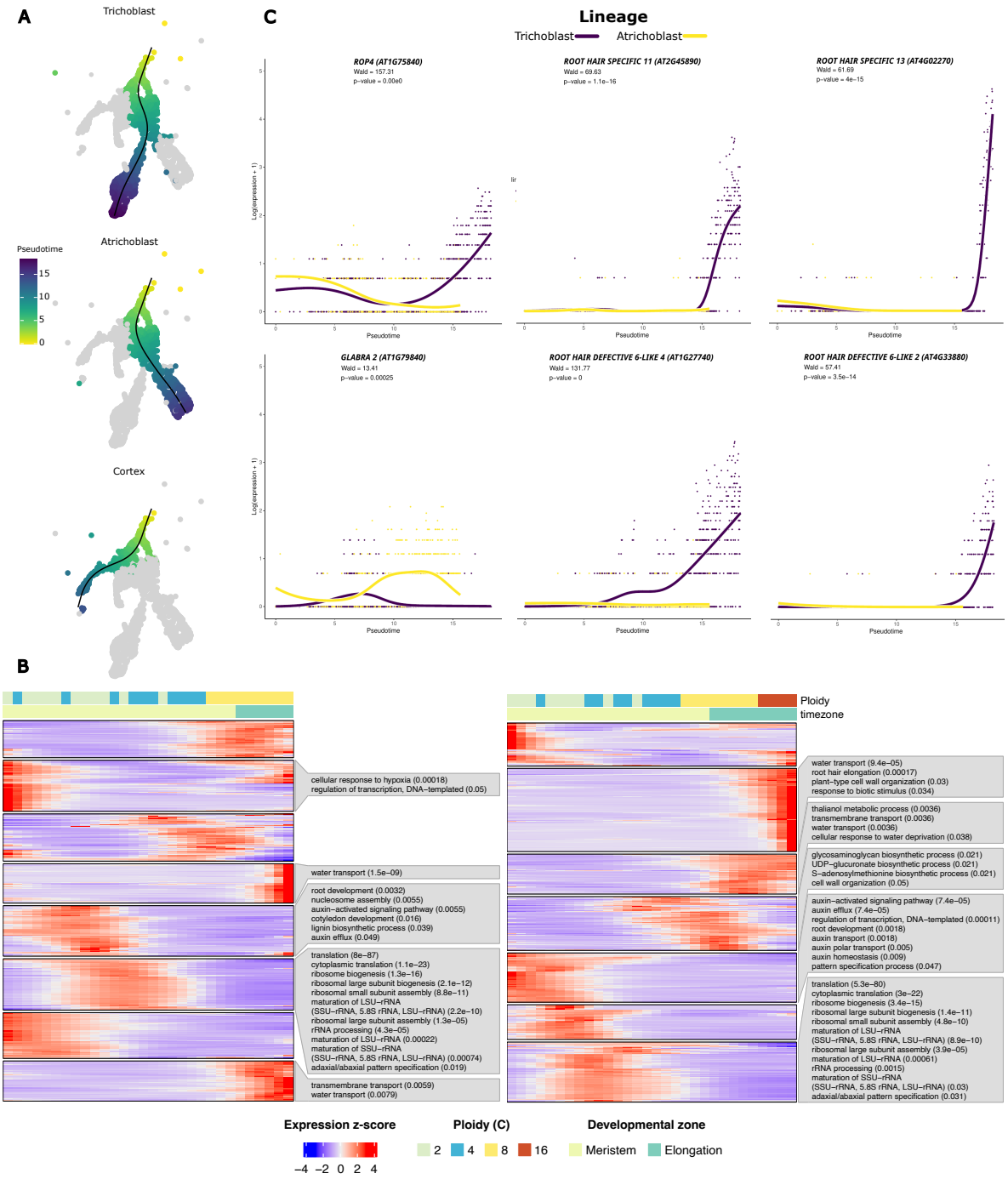

Figure 2: Differential expression of root hair growth regulators across pseudotime. (A) UMAP describing the developmental lineages from a central cluster (QC/meristematic cells) to differentiated trichoblast, atrichoblast and cortex cell types. The lineages were identified via Slingshot; the black line 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.

2 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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