© 2022. Published by The Company of Biologists Ltd | Development (2022) 149, dev200179. doi:10.1242/dev.200179

SPOTLIGHT

Single-cell genomics revolutionizes plant development studies across scales

Mingyuan Zhu[1,*], Isaiah W. Taylor[1,*] and Philip N. Benfey[1,2,‡]

ABSTRACT

Understanding the development of tissues, organs and entire organisms through the lens of single-cell genomics has revolutionized developmental biology. Although single-cell transcriptomics has been pioneered in animal systems, from an experimental perspective, plant development holds some distinct advantages: cells do not migrate in relation to one another, and new organ formation (of leaves, roots, flowers, etc.) continues post-embryonically from persistent stem cell populations known as meristems. For a time, plant studies lagged behind animal or cell culture-based, single-cell approaches, largely owing to the difficulty in dissociating plant cells from their rigid cell walls. Recent intensive development of single-cell and single-nucleus isolation techniques across plant species has opened up a wide range of experimental approaches. This has produced a rapidly expanding diversity of information across tissue types and species, concomitant with the creative development of methods. In this brief Spotlight, we highlight some of the technical developments and how they have led to profiling single-cell genomics in various plant organs. We also emphasize the contribution of single-cell genomics in revealing developmental trajectories among different cell types within plant organs. Furthermore, we present efforts toward comparative analysis of tissues and organs at a single-cell level. Single-cell genomics is beginning to generate comprehensive information relating to how plant organs emerge from stem cell populations.

KEY WORDS: *Arabidopsis*, Plant, Plant development, Single cell

Introduction

Plants have a remarkable ability to adaptively generate new organs. For instance, in contrast to animals, in which clonal propagation typically requires highly sophisticated laboratory techniques, plants often require no more than a simple stem cutting to regenerate an entirely new individual (Hussain et al., 2012). Understanding the principles of organ development and function in plants that underlie this phenomenon could address fundamental questions in developmental biology relating to differentiation dynamics in complex multicellular organisms. At the same time, these insights into mechanisms of growth will be crucial for adapting agriculture to climate change and population pressure.

Organ development in all organisms is directly tied to the pattern of transcriptional regulation within cells. A paradigm shift in developmental biology has occurred over the last decade as technical progress has enabled gene expression measurements at single-cell resolution with increasing ease and throughput. In plants, this adoption has been slowed by the difficulty in dissociating plant cells from their encapsulating cell walls. Methods have had to be optimized for single-cell dissociation for the many plant species under study. The result has been a flood of recent publications employing single-cell techniques across a wide range of tissues and species. In this Spotlight, we highlight the technical progress enabling these discoveries, along with studies of developmental dynamics and comparative genomics at the single-cell level. The advance of single-cell genomics in plants has heightened our understanding of organ formation and function.

Single-cell RNA sequencing in plants is rapidly advancing

Single-cell RNA-sequencing (scRNA-seq) studies in plants generally share the same basic workflow: a single-cell (or single-nucleus) suspension is generated, cells are separated into distinct compartments (either droplets of an emulsion or wells of a plate) containing barcoded reverse transcriptase (RT) primers (Fig. 1A). Typically, random nucleotide sequences, known as unique molecular identifiers (UMIs) are included on the RT primers to tag uniquely each individual first strand cDNA molecule, which enables subsequent identification and removal of PCR duplicated reads during the analysis stage. The resulting cDNA is used to generate a library for sequencing, after which sequenced reads are grouped according to shared barcodes (Fig. 1B). Finally, downstream analyses are performed to identify cell types, etc. (Fig. 1C). In plants, the primary complication is in the dissociation of cells embedded in a rigid, chemically stable cell wall. Until recently, enzymatic digestion to generate suspensions of plant cells lacking cell walls (protoplasts) was employed infrequently in developmental studies. An exception is the characterization of cell types of the *Arabidopsis* root, which are relatively simple to protoplast, and the root has long been a model developmental system. Studies of the *Arabidopsis* root were the first single-cell analyses published in plants and demonstrated a phenomenal ability to identify cell types reflecting known biological reality (Denyer et al., 2019; Efroni et al., 2016; Jean-Baptiste et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019).

Following the success with *Arabidopsis* roots, there has been a surge in developing protoplasting techniques. Single-cell analyses have now been performed on protoplasts derived from a wide range of species and tissue types by modifying parameters, such as enzyme composition, concentration and/or infiltration conditions, digestion temperature and length, and tissue dissection (Fig. 1D). Beyond the basic technique of protoplasting, which involves coarsely chopping tissue and immersing in digestion solution, additional enabling techniques are being developed. For instance, Marchant and colleagues have recently demonstrated that chemical fixation of tissue (thus halting cellular metabolism), followed by digestion with highly pure, RNase-free, cell wall-digesting enzymes

[1]Department of Biology, Duke University, Durham, NC 27708, USA. [2]Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA.
*These authors contributed equally to this work

‡Author for correspondence (philip.benfey@duke.edu)

M.Z., 0000-0003-1237-2760; I.W.T., 0000-0002-6535-8658; P.N.B., 0000-0001-5302-758X

Development (2022) 149, dev200179. doi:10.1242/dev.200179

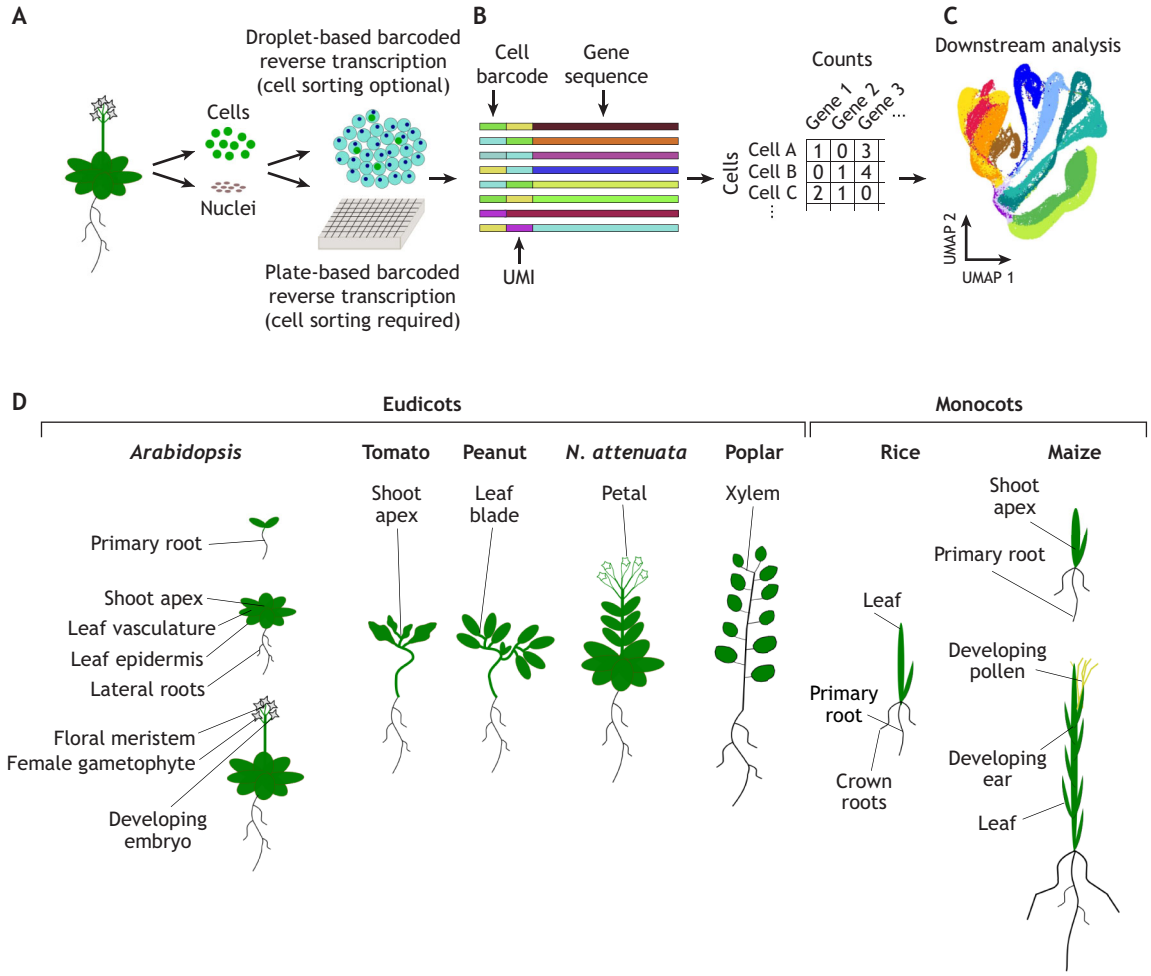

Fig. 1. Overview of plant single-cell studies. (A-C) General workflow for taking an intact plant and isolating cells or nuclei, before generating barcoded cDNA, leading to a final count matrix. (D) Example species/tissues that have been profiled by single-cell RNA sequencing (Denyer et al., 2019; Gala et al., 2021; Jean-Baptiste et al., 2019; Liu et al., 2020; Lopez-Anido et al., 2021; Ryu et al., 2019; Serrano-Ron et al., 2021; Shahan et al., 2022; Shulse et al., 2019; Zhang et al., 2019). *N. attenuata*, *Nicotiana attenuata*.

at an elevated temperature, vastly improves yields of protoplasts from maize anthers and root tips (Marchant et al., 2021 preprint). Other groups have used mechanical means to expose the cell population of interest. For instance, Kim and colleagues have used a 'tape sandwich' technique (Wu et al., 2009) to remove epidermal cells of *Arabidopsis* leaves and facilitate protoplast release of the underlying vasculature (Kim et al., 2021), whereas Li and colleagues have scraped the bark off poplar stem segments to expose the outer xylem (Li et al., 2021). As a result of these intense optimizations, there is now a rich set of validated techniques across systems that can be used as a starting point for protocol development.

Once optimized, protoplasting is a relatively simple technique and produces highly informative, validated insights. However, it is not without drawbacks. A major issue is that cell types have differing protoplasting efficiency, leading to skewed distributions of cell types. For instance, protoplasting of maize leaves has not recovered any putative vascular cells (Bezrutczyk et al., 2021). An alternative approach is to isolate nuclei that contain sufficient quantities of mRNA for analysis. Isolation of nuclei involves mechanical disruption, such as fine chopping of tissue with a razor blade, to break open the cell walls. This technique has a number of potential benefits over protoplasting, including enhanced recovery of difficult-to-protoplast cells, the possibility of freezing or fixing tissue prior to isolation (which is essential for analyzing field-grown plants), and greatly reduced transcriptomic shock associated with isolation (protoplast digestion typically lasts 1-2 h) (Farmer et al., 2021; Picard et al., 2021; Sunaga-Franze et al., 2021; Tian et al., 2020 preprint). However, mRNA yield from nuclei is substantially lower and nuclei are susceptible to damage and/or RNA leakage. In contrast to protoplasts, for which cell viability can be determined by visual parameters, it is harder to assess the quality of a nucleus preparation. These drawbacks notwithstanding, because nucleus-based approaches still have sufficient advantages over protoplasting, they are likely to play an increasing role in the future of plant single-cell research.

Although computational analysis tools of single-cell data are not the focus of this article, standard scRNA-seq software packages, such as Seurat (Stuart et al., 2019) and Monocle (Trapnell et al., 2014), have yielded reasonable and validated clustering of cells and maps of developmental dynamics. The most common way to visualize clusters of cells is by uniform manifold approximation and projection (UMAP), which is a non-linear projection of data into low dimensional space (Fig. 1C). The first analytical method development papers specifically employing plant data are beginning to appear (Yan et al., 2020 preprint). Once putative cell

Development (2022) 149, dev200179. doi:10.1242/dev.200179

types have been identified, validation has relied on a combination of comparison with expression patterns of published marker genes (mostly in *Arabidopsis*), *in situ* hybridization and the generation of fluorescent reporter lines. In a recent example of targeted cell isolation, Ortiz-Ramírez and colleagues have used the geometry of maize roots and a novel differential dye penetration ratiometric technique combined with fluorescence-activated cell sorting to isolate cells with concentric radii, highlighting the creative possibilities inherent in this new technology (Ortiz-Ramírez et al., 2021).

Developmental trajectory analysis provides insights into cell differentiation

Cell-type clustering together with annotation is a fundamental step in the analysis of scRNA-seq data. Clustering relies on reducing the dimensionality of the expression data matrix, and proper annotation leads to the analysis of specific cell types. The gene expression data of individual cells not only indicates their cell type or fate, but also provides clues regarding their developmental stage. Differences in gene expression between cells can be used to infer their developmental order. For developmental trajectory analysis, there are two major objectives: (1) determine the developmental order of distinct cell groups; and (2) identify the important molecular events at each step of differentiation.

When specific cell types within the tissue sample are of particular interest, it is often advantageous to increase their representation in the pool of cells sequenced. Researchers have managed to harvest high-quality cells for scRNA-seq by manual isolation from maize anthers (Nelms and Walbot, 2019), maize shoot apex (Satterlee et al., 2020), *Arabidopsis* phloem (Roszak et al., 2021 preprint), *Arabidopsis* leaf epidermis (Lopez-Anido et al., 2021) and *Arabidopsis* lateral root primordia (Serrano-Ron et al., 2021). Owing to the highly enriched target cells, hundreds of cells are often sufficient for single-cell analyses. However, tissue dissection or cell sorting is labor intensive and requires expertise. To overcome such technical difficulties without sacrificing specificity, Gala and colleagues have used gravistimulation to synchronize the formation of lateral root primordia and then harvested the region of interest (Gala et al., 2021). Although this experimental design requires developmental events that are easily triggered, it highlights the possibility of combining an inducible system with scRNA-seq. Another option is to use specific markers to filter cells bioinformatically after generating gene expression data for all cells across a tissue. The filtering can be based on either expression of known markers or the expression of a transgene with a validated expression pattern. For example, Hou and colleagues separated the clusters relevant to female germline development (3 of 28 clusters) based on the expression of a megaspore mother cell fluorescent marker (Hou et al., 2021). This type of filtering facilitates sample preparation, although it requires knowledge of known markers and a much bigger sample size (thousands of cells), which often includes a large proportion of irrelevant cells. As the cost of library construction and sequencing decreases, bioinformatic filtering methods should become more advantageous because it avoids cell stress caused by cell sorting.

Separating cells of a certain cell type and treating them as bulk data allows the identification of gene expression characteristics. However, the developmental stage of the cells remains unclear. Pseudotime trajectory analysis enables the placement of cells in a defined developmental order based on their gene expression. Typically, the less differentiated cells are placed at the beginning of cell fate progressions whereas the more differentiated cells are placed at the periphery of the cluster cloud. Pseudotime analyses can be performed with various packages available online, such as Monocle 2/3 (Cao et al., 2019; Trapnell et al., 2014), velocyto (la Manno et al., 2018), CytoTRACE (Gulati et al., 2020) and Palantir (Setty et al., 2019) (Fig. 2A). By comparing the developmental

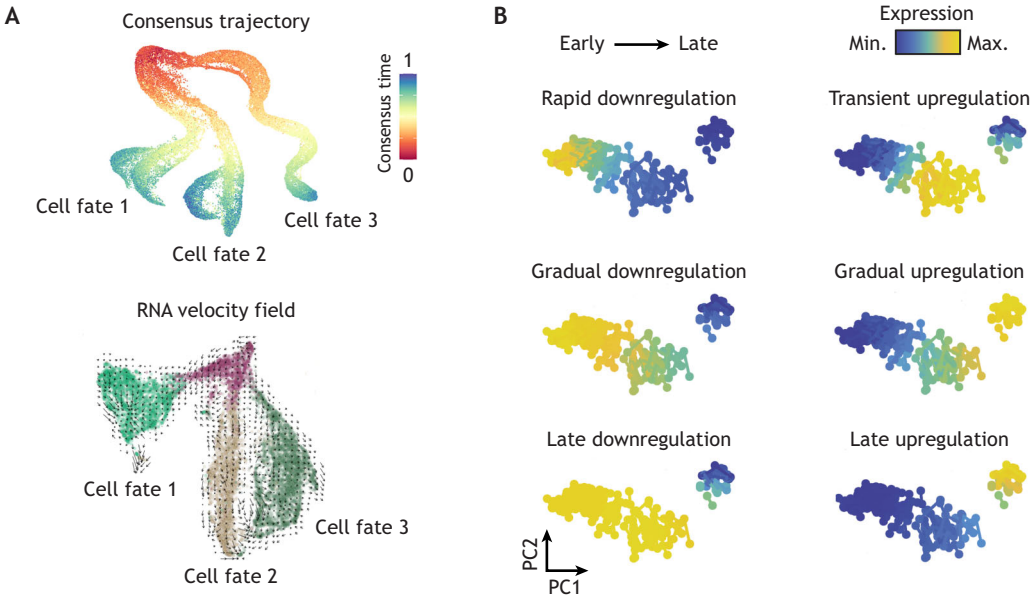

Fig. 2. Pseudotime analyses of cell differentiation trajectories. (A) Pseudotime analyses can capture developmental trajectories. Top: A consensus time heat map infers the developmental state of each cell. Warmer colors denote younger cells whereas cooler colors denote older cells (adapted from Shahan et al., 2022). Bottom: RNA velocity field projected onto the UMAP. Different colors indicate different cell types. Arrows represent average velocity and differentiation direction (adapted from Zhang et al., 2021b). (B) Various patterns for gene expression dynamics. Gene expression change could happen at an early stage (e.g. rapid downregulation; top), an intermediate stage (e.g. gradual downregulation and upregulation; middle) and a late stage (e.g. late downregulation and upregulation; bottom). Rapid upregulation and transient downregulation gene expression patterns are not pictured here because of fewer representative genes in the developmental program of maize male meiosis. Adapted from Nelms and Walbot (2019).

Development (2022) 149, dev200179. doi:10.1242/dev.200179

trajectories predicted by pseudotime with previous experimentally characterized data, pseudotime trajectory analyses of selected clusters faithfully recapitulate the cell differentiation of ground tissue (Jean-Baptiste et al., 2019; Shahan et al., 2022; Shulse et al., 2019; Zhang et al., 2021b), stomata (Liu et al., 2020; Lopez-Anido et al., 2021; Zhang et al., 2021a), root cap (Zhang et al., 2019), lateral root (Gala et al., 2021; Serrano-Ron et al., 2021), vascular tissue (Roszak et al., 2021 preprint; Tian et al., 2020 preprint), female germ cells (Hou et al., 2021), root hairs (Denyer et al., 2019; Ryu et al., 2019; Zhang et al., 2021b), mesophyll cells (Wang et al., 2021) and trichomes (Tian et al., 2020 preprint). For less-characterized cell lineages or for species lacking comprehensive genome annotation, pseudotime analyses can contribute to cluster annotation. By integrating marker gene expression, UMAP clustering and cell distributions by pseudotime analyses, Li and colleagues have been able to define the cell types of six clusters relevant to the differentiation of fiber cells and ray parenchyma cells in *Populus* (Li et al., 2021). The precise capture of cell types along a developmental trajectory reveals the power of single-cell genomics to elucidate the developmental progression of plant organs.

In addition to validating cell lineage and cluster annotation, pseudotime analyses of scRNA-seq data also facilitate the study of gene expression dynamics throughout differentiation. Gene expression along a pseudotime trajectory can be categorized into eight major patterns: rapid downregulation/upregulation (early stage), gradual downregulation/upregulation, transient downregulation/upregulation (intermediate stage) and late downregulation/upregulation (late stage) (Fig. 2B). Genes with distinct expression patterns are often annotated to different gene ontology categories, which indicates that they are involved in different pathways. Rapid change at early stages is often linked to meristem homeostasis. Transient up- or downregulation can be relevant to the transition between differentiation steps. Late expression changes indicate potential roles as terminal cell fate regulators. For example, the expression of rNTP-biosynthesis-relevant genes exhibit rapid downregulation during germinal differentiation (Nelms and Walbot, 2019). *KNOTTED1* (*KN1*), a key marker of maize indeterminate meristematic identity, falls into the gradual downregulation category (Satterlee et al., 2020). Photosynthesis genes show late upregulated expression in the tomato shoot apex mesophyll branch, indicating their role in the terminal stage of differentiation (Tian et al., 2020 preprint). In addition, pseudotime analyses have enabled the identification of new regulators of cell differentiation. Searching for genes with enriched expression in the xylem lineage identified an auxin-responsive gene, *AT2G04850*, in which mutations lead to fewer xylem cells in the *Arabidopsis* hypocotyl (Zhang et al., 2021a). Comparisons of gene expression between different stages of lateral root differentiation have revealed a previously unknown link between various auxin signaling-related genes and lateral root initiation (Gala et al., 2021). Furthermore, pseudotime analyses allow the comparison of overall transcriptional patterning at the single-cell level along the developmental trajectory. Indeed, Lopez-Anido and colleagues have shown that single-cell transcriptional diversity peaks in *Arabidopsis* guard mother cells during the middle stages of stomata differentiation rather than prior to differentiation commitment, which is common in animals (Lopez-Anido et al., 2021).

By studying developmental trajectories with scRNA-seq data, insights have been gained into cell lineage, gene expression dynamics and transcript heterogeneity at the cellular level. Cells within a tissue are not only spatially arranged, but also temporally connected in a developmental continuum.

Comparative single-cell genomics illuminates diversity and conservation at various scales

Pooling for bulk RNA sequencing (RNA-seq) generates sufficient information to determine gene expression levels. However, changes in transcript levels in bulk RNA-seq only reflect the average behavior and lack cell-to-cell variation. scRNA-seq reveals the heterogeneity between cells, thus enabling a comparison between samples at a finer scale.

Once single-cell techniques have been established for a specific organ, this enables the comparison of gene expression at the single-cell level for the same organ among different genetic backgrounds and growth conditions. The detection of cell type-specific expression changes between wild-type and mutant lines have been performed in *Arabidopsis* for mutations affecting ground tissue (Shahan et al., 2022), root hairs (Ryu et al., 2019) and brassinosteroid signaling (Graeff et al., 2021). The comparison between wild type and mutants can identify the effects of genetic mutations on cell-type specification. Interestingly, based on scRNA-seq data from the *Arabidopsis scarecrow* mutant, some cortex-like cells at early stages change their fate to more endodermis-like cells in the maturation zone, which cannot be detected with bulk RNA-seq (Shahan et al., 2022). Comparison of cell populations and gene expression has been performed for *Arabidopsis* roots undergoing heat stress (Jean-Baptiste et al., 2019) and growing on sucrose (Shulse et al., 2019), as well as rice seedlings under high/low salinity or high/low nitrogen conditions (Wang et al., 2021), and *Chlamydomonas* with or without iron deficiency or nitrogen deficiency (Ma et al., 2021). Compared with bulk RNA-seq, scRNA-seq enables more efficient detection of cell differentiation patterns. For example, scRNA-seq analyses have indicated that abiotic stress alters the pace of differentiation for mesophyll cells in rice seedlings (Wang et al., 2021). Overall, the trends in the modulation of gene expression with perturbation are relatively conserved: (1) the direction in the change of gene expression in scRNA-seq data is consistent with that found in bulk RNA-seq; (2) genetic mutations and abiotic stress often alter the proportion of relevant cell types; and (3) different cell types respond differentially to abiotic stress, although relevant genetic pathways are relatively conserved.

Comparison of single-cell genomics at various scales has allowed researchers to compare gene expression and chromatin accessibility across different organs and species (Fig. 3). Wang and colleagues have applied scRNA-seq to both shoots and roots of rice seedlings (Wang et al., 2021) and found that common transcriptome features are shared between cell groups with similar tissue positioning (i.e. leaf mestome sheath and root pericycle, both the outermost cell layer of the vascular cylinder) and function (e.g. leaf phloem and root phloem). In contrast, gene expression patterns are not conserved for the epidermis of roots and shoots owing to the dramatically distinct environments to which they are exposed. Comparison of gene expression between different organs has suggested the possible existence of conserved regulatory mechanisms. For example, the observation that *ARABIDOPSIS THALIANA MERISTEM LAYER 1* (*ATML1*) is strongly expressed in both the root and shoot epidermis led to further experimentation that revealed a previously unknown role for ATML1 in cuticle biosynthesis in roots (Zhang et al., 2021a). Thus, conserved gene expression patterns among different organs can reflect their morphological or functional similarities (Fig. 3A). scRNA-seq has also been performed for two rice cultivars: *japonica* group cultivar (Nipponbare) and *indica* group cultivar (93-11) (Liu et al., 2021). Eight annotated cell-type clusters have been well matched between the two cultivars. The greatest

Development (2022) 149, dev200179. doi:10.1242/dev.200179

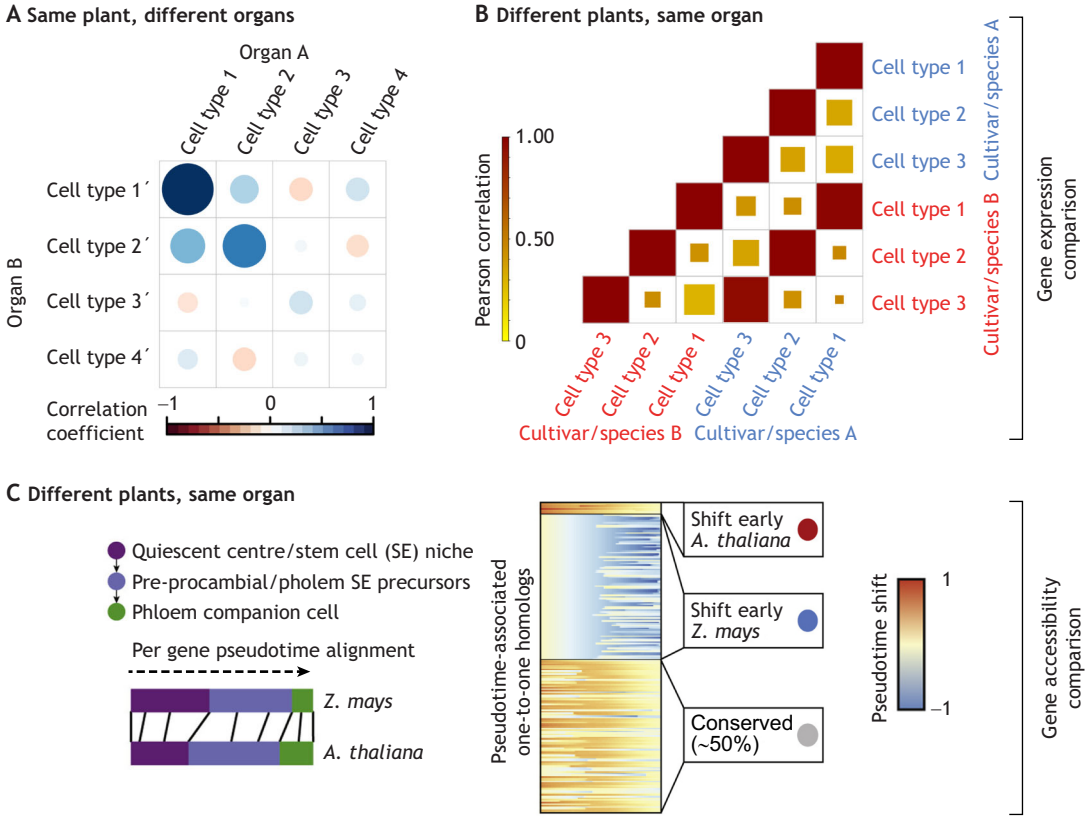

Fig. 3. Comparative single-cell studies reveal genomic conservation among various tissues, cultivars and species. (A) The pairwise Spearman's correlation coefficients for the expression profile between different organs in the same individual plant. Hypothetical cell types 1 and 1′ demonstrate a similar anatomical position and function (experimental example: root phloem and leaf phloem); cell types 2 and 2′ demonstrate a different anatomical position but similar function (experimental example: root endodermis and leaf initial cells); cell types 3 and 3′ demonstrate a similar anatomical position but different function (experimental example: root epidermis and leaf epidermis); and cell types 4 and 4′ demonstrate a different anatomical position and function. Color scale: blue, positive correlation; red, negative correlation; white, no correlation. Areas of circles present the absolute value of corresponding correlation coefficients. More detailed experimental comparison between rice roots and leaves can be found in Wang et al. (2021). (B) Simplified Pearson correlation heatmap comparing cell-type transcriptomes between different cultivars or species. Cell-type homologies between hypothetically similar organs (i.e. cell type 1 in cultivar/species A versus cell type 1 in cultivar/species B) are supported by the high Pearson's correlation coefficients of the expression profiles between cell-type clusters (experimental example: rice *japonica* Nip root cortex versus rice *indica* 93-11 root cortex). Color scale: red, high correlation; yellow, low correlation. Areas of squares represent the absolute value of corresponding correlation coefficients. More detailed experimental comparison between rice *japonica* and *indica* cell types can be found in Liu et al. (2021). (C) Left: Developmental trajectory of root phloem companion cell (PCC; top) and global alignment of gene accessibility for individual orthologs is performed across *A. thaliana* and *Zea mays* PCC pseudotime trajectories (bottom). Right: PCC development-relevant orthologs are clustered into three groups based on pseudotime shifts, which represent the extent of gene accessibility deviation at any given point along the trajectory Although only around 2% of orthologs are associated with PCC pseudotime in both species, ~50% of these orthologs exhibit similar gene accessibility patterns across pseudotime, indicating highly conserved gene accessibility pattern for genes with conserved functions (adapted from Marand et al., 2021).

difference was observed between genes expressed in the root cap, which mirrors the dramatic morphological difference between root caps of the two subspecies. The developmental trajectories of various cell types are also conserved between the two rice cultivars, indicating that not only the final cell fates but also the developmental order is conserved (Fig. 3B).

Comparative transcriptomics has also provided insights into cell functions between different species. *Arabidopsis* and rice roots share similar gene expression patterns for some cell types (i.e. stem cell niche, epidermis and vascular tissue). It is also notable that the rice exodermis has similar transcriptomics to the *Arabidopsis* endodermis, reflecting the fact that both serve as intercellular transport barriers (Zhang et al., 2021b). Cells of the *Arabidopsis* cortex can be aligned with a subgroup of rice cortex cells, also reflecting a partially conserved function (Liu et al., 2021). Similar to the comparison between two organs within the same species, conservation of gene expression across different species is correlated with morphological and functional similarities. As techniques advance, it is now possible to track chromatin accessibility with single-cell sequencing of assay for transposase accessible chromatin (scATAC-seq). Single-cell or single-nucleus ATAC-seq has been applied to *Arabidopsis* roots (Dorrity et al., 2021; Farmer et al., 2021). ATAC-seq at the single-cell level is used to infer cell type-specific patterns of chromatin accessibility. Integration of scRNAseq and scATAC-seq has revealed that both transcriptional diversity and chromatin landscape are associated with developmental stages (reviewed by Marand and Schmitz, 2022). Consequently, chromatin accessibility has become another genomic parameter for comparison. When chromatin accessibility resolution has been compared between *Arabidopsis* and maize, a high degree of conservation in the cis-regulatory specification of phloem companion cell development has been revealed (Marand et al., 2021) (Fig. 3C). Work combining scRNAseq and scATACseq in two distinct species provides a framework to identify transcription factors necessary for the evolutionarily conserved development of plant tissues.

Development (2022) 149, dev200179. doi:10.1242/dev.200179

Concluding remarks and future perspectives

Breakthroughs in single-cell genomics have enabled us to understand plant development at an unprecedented level of detail with more and more organs in various plant species now being studied. The results have validated previously published cell-type markers and cell-differentiation trajectories. Furthermore, they provide deep insights into cell-type regulators and differentiation pathways. Beyond the study of single plant organs, comparative studies of gene expression at the single-cell level are revealing transcriptional diversity and conservation across organs and species.

There are several challenges and opportunities for the plant research community to achieve a more comprehensive and precise understanding of the gene regulatory networks underlying plant organ development at single-cell resolution. First, the use of spatial transcriptomics can capture the expression of hundreds of genes while retaining their positional context within a tissue. Spatial transcriptomics provides direct spatial information on gene expression compared with that inferred from scRNA-seq. Researchers have recently attempted to conduct spatial transcriptomics on the maize shoot apex (Laureyns et al., 2021) and have applied related techniques (e.g. DNA nanoball) to *Arabidopsis* leaves (Xia et al., 2021 preprint). Integrative single-cell analyses are another exciting frontier. The comparison between scRNA-seq and scATACseq data in maize has shown that chromatin accessibility provides additional information for determining cell types and stages (Marand et al., 2021). The combination of scRNA-seq, scATAC-seq and potential single-cell proteomics will enable the study of gene expression and cell function at several molecular scales. 'Combinatorial indexing', whereby cells/nuclei are barcoded at multiple points in the experiments, promise to increase throughput dramatically by allowing more cell/nuclei to be sequenced in one experiment (Cao et al., 2017), and will allow the introduction of more intrinsic gene perturbation and extrinsic biotic/abiotic stress into the scRNA-seq experimental design. Together, this approach will broaden our understanding of the cellular response to developmental defects and stress. Lastly, there are more and more single-cell genomics data being published, which makes the standardization of data processing and accessibility an urgent issue. Efforts are already underway (Chen et al., 2021; Rhee et al., 2019), but coordination throughout the plant biology community is highly encouraged.

Acknowledgements

We apologize to colleagues whose work was not cited due to space constraints. We would like to acknowledge Dr Adrienne Roeder and Dr Lilan Hong for critical reading of the manuscript.

Competing interests

P.N.B. is the co-founder and Chair of the Scientific Advisory Board of Hi Fidelity Genetics, Inc., a company that works on crop root growth.

Funding

This work was funded by grants from the National Science Foundation (PHY-1915445) and the National Institutes of Health (MIRA 1R35GM131725), and by the Howard Hughes Medical Institute to P.N.B. as an Investigator. I.W.T. was supported by the U.S. Department of Agriculture-National Institute of Food and Agriculture (2021-67034-35139). Deposited in PMC for release after 12 months.

References

Bezrutczyk, M., Zöllner, N. R., Kruse, C. P. S., Hartwig, T., Lautwein, T., Köhrer, K., Frommer, W. B. and Kim, J.-Y. (2021). Evidence for phloem loading via the abaxial bundle sheath cells in maize leaves. *Plant Cell* **33**, 531-547. doi:10.1093/plcell/koaa055

Cao, J., Packer, J. S., Ramani, V., Cusanovich, D. A., Huynh, C., Daza, R., Qiu, X., Lee, C., Furlan, S. N., Steemers, F. J. et al. (2017). Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science* **357**, 661-667. doi:10.1126/science.aam8940

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F. J. et al. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496-502. doi:10.1038/s41586-019-0969-x

Chen, H., Yin, X., Guo, L., Yao, J., Ding, Y., Xu, X., Liu, L., Zhu, Q.-H., Chu, Q. and Fan, L. (2021). PlantscRNAdb: a database for plant single-cell RNA analysis. *Mol. Plant* **14**, 855-857. doi:10.1016/j.molp.2021.05.002

Denyer, T., Ma, X., Klesen, S., Scacchi, E., Nieselt, K. and Timmermans, M. C. P. (2019). Spatiotemporal developmental trajectories in the Arabidopsis root revealed using high-throughput single-cell RNA sequencing. *Dev. Cell* **48**, 840-852.e5. doi:10.1016/j.devcel.2019.02.022

Dorrity, M. W., Alexandre, C. M., Hamm, M. O., Vigil, A.-L., Fields, S., Queitsch, C. and Cuperus, J. T. (2021). The regulatory landscape of Arabidopsis thaliana roots at single-cell resolution. *Nat. Commun.* **12**, 1-12. doi:10.1038/s41467-021-23675-y

Efroni, I., Mello, A., Nawy, T., Ip, P.-L., Rahni, R., Delrose, N., Powers, A., Satija, R. and Birnbaum, K. D. (2016). Root regeneration triggers an embryo-like sequence guided by hormonal interactions. *Cell* **165**, 1721-1733. doi:10.1016/j.cell.2016.04.046

Farmer, A., Thibivilliers, S., Ryu, K. H., Schiefelbein, J. and Libault, M. (2021). Single-nucleus RNA and ATAC sequencing reveals the impact of chromatin accessibility on gene expression in Arabidopsis roots at the single-cell level. *Mol. Plant* **14**, 372-383. doi:10.1016/j.molp.2021.01.001

Gala, H. P., Lanctot, A., Jean-Baptiste, K., Guiziou, S., Chu, J. C., Zemke, J. E., George, W., Queitsch, C., Cuperus, J. T. and Nemhauser, J. L. (2021). A single-cell view of the transcriptome during lateral root initiation in Arabidopsis thaliana. *Plant Cell* **33**, 2197-2220. doi:10.1093/plcell/koab101

Graeff, M., Rana, S., Wendrich, J. R., Dorier, J., Eekhout, T., Aliaga Fandino, A. C., Guex, N., Bassel, G. W., de Rybel, B. and Hardtke, C. S. (2021). A single-cell morpho-transcriptomic map of brassinosteroid action in the Arabidopsis root. *Mol. Plant* **14**, 1985-1999. doi:10.1016/j.molp.2021.07.021

Gulati, G. S., Sikandar, S. S., Wesche, D. J., Manjunath, A., Bharadwaj, A., Berger, M. J., Ilagan, F., Kuo, A. H., Hsieh, R. W., Cai, S. et al. (2020). Single-cell transcriptional diversity is a hallmark of developmental potential. *Science* **367**, 405-411. doi:10.1126/science.aax0249

Hou, Z., Liu, Y., Zhang, M., Zhao, L., Jin, X., Liu, L., Su, Z., Cai, H. and Qin, Y. (2021). High-throughput single-cell transcriptomics reveals the female germline differentiation trajectory in Arabidopsis thaliana. *Commun. Biol.* **4**, 1149. doi:10.1038/s42003-021-02676-z

Hussain, A., Qarshi, I. A., Nazir, H. and Ullah, I. (2012). Plant tissue culture: current status and opportunities. *Recent Adv. Plant in vitro Culture* **6**, 1-28. doi:10.5772/50568

Jean-Baptiste, K., McFaline-Figueroa, J. L., Alexandre, C. M., Dorrity, M. W., Saunders, L., Bubb, K. L., Trapnell, C., Fields, S., Queitsch, C. and Cuperusa, J. T. (2019). Dynamics of gene expression in single root cells of arabidopsis thaliana. *Plant Cell* **31**, 993-1011. doi:10.1105/tpc.18.00785

Kim, J.-Y., Symeonidi, E., Pang, T. Y., Denyer, T., Weidauer, D., Bezrutczyk, M., Miras, M., Zöllner, N., Hartwig, T., Wudick, M. M. et al. (2021). Distinct identities of leaf phloem cells revealed by single cell transcriptomics. *Plant Cell* **33**, 511-530. doi:10.1093/plcell/koaa060

la Manno, G., Soldatov, R., Zeisel, A., Braun, E., Hochgerner, H., Petukhov, V., Lidschreiber, K., Kastriti, M. E., Lönnerberg, P., Furlan, A. et al. (2018). RNA velocity of single cells. *Nature* **560**, 494-498. doi:10.1038/s41586-018-0414-6

Laureyns, R., Joossens, J., Herwegh, D., Pevernagie, J., Pavie, B., Demuynck, K., Debray, K., Coussens, G., Pauwels, L., van Hautegem, T. et al. (2021). An in situ sequencing approach maps PLASTOCHRON1 at the boundary between indeterminate and determinate cells. *Plant Physiol.* **188**, 782-794. doi:10.1093/plphys/kiab533

Li, H., Dai, X., Huang, X., Xu, M., Wang, Q., Yan, X., Sederoff, R. R. and Li, Q. (2021). Single-cell RNA sequencing reveals a high-resolution cell atlas of xylem in Populus. *J. Integr. Plant Biol.* **63**, 1906-1921. doi:10.1111/jipb.13159

Liu, Z., Zhou, Y., Guo, J., Li, J., Tian, Z., Zhu, Z., Wang, J., Wu, R., Zhang, B., Hu, Y. et al. (2020). Global dynamic molecular profiling of stomatal lineage cell development by single-cell RNA sequencing. *Mol. Plant* **13**, 1178-1193. doi:10.1016/j.molp.2020.06.010

Liu, Q., Liang, Z., Feng, D., Jiang, S., Wang, Y., Du, Z., Li, R., Hu, G., Zhang, P., Ma, Y. et al. (2021). Transcriptional landscape of rice roots at the single-cell resolution. *Mol. Plant* **14**, 384-394. doi:10.1016/j.molp.2020.12.014

Lopez-Anido, C. B., Vatén, A., Smoot, N. K., Sharma, N., Guo, V., Gong, Y., Anleu Gil, M. X., Weimer, A. K. and Bergmann, D. C. (2021). Single-cell resolution of lineage trajectories in the Arabidopsis stomatal lineage and developing leaf. *Dev. Cell* **56**, 1043-1055.e4. doi:10.1016/j.devcel.2021.03.014

Ma, F., Salomé, P. A., Merchant, S. S. and Pellegrini, M. (2021). Single-cell RNA sequencing of batch Chlamydomonas cultures reveals heterogeneity in their diurnal cycle phase. *Plant Cell* **33**, 1042-1057. doi:10.1093/plcell/koab025

Marand, A. P. and Schmitz, R. J. (2022). Single-cell analysis of cis-regulatory elements. *Curr. Opin. Plant Biol.* **65**, 102094. doi:10.1016/j.pbi.2021.102094

Marand, A. P., Chen, Z., Gallavotti, A. and Schmitz, R. J. (2021). A cis-regulatory atlas in maize at single-cell resolution. *Cell* **184**, 3041-3055.e21. doi:10.1016/j.cell.2021.04.014

Marchant, D. B., Nelms, B. and Walbot, V. (2021). Quantitative cell release from plant tissues for single-cell genomics. *bioRxiv* doi:10.1101/2021.10.11.463960

Nelms, B. and Walbot, V. (2019). Defining the developmental program leading to meiosis in maize. *Science* **364**, 52-56. doi:10.1126/science.aav6428

Ortiz-Ramírez, C., Guillotin, B., Xu, X., Rahni, R., Zhang, S., Yan, Z., Coqueiro Dias Araujo, P., Demesa-Arevalo, E., Lee, L., van Eck, J. et al. (2021). Ground tissue circuitry regulates organ complexity in maize and Setaria. *Science* **374**, 1247-1252. doi:10.1126/science.abj2327

Picard, C. L., Povilus, R. A., Williams, B. P. and Gehring, M. (2021). Transcriptional and imprinting complexity in Arabidopsis seeds at single-nucleus resolution. *Nat. Plants* **7**, 730-738. doi:10.1038/s41477-021-00922-0

Rhee, S. Y., Birnbaum, K. D. and Ehrhardt, D. W. (2019). Towards building a plant cell atlas. *Trends Plant Sci.* **24**, 303-310. doi:10.1016/j.tplants.2019.01.006

Roszak, P., Heo, J., Blob, B., Toyokura, K., Angels de Luis Balaguer, M., Lau, W. W., Hamey, F., Cirrone, J., Wang, X., Ursache, R. et al. (2021). Analysis of phloem trajectory links tissue maturation to cell specialization. *bioRxiv*. doi:10.1101/2021.01.18.427084

Ryu, K. H., Huang, L., Kang, H. M. and Schiefelbein, J. (2019). Single-cell RNA sequencing resolves molecular relationships among individual plant cells. *Plant Physiol.* **179**, 1444-1456. doi:10.1104/pp.18.01482

Satterlee, J. W., Strable, J. and Scanlon, M. J. (2020). Plant stem-cell organization and differentiation at single-cell resolution. *Proc. Natl Acad. Sci. USA* **117**, 33689-33699. doi:10.1073/pnas.2018788117

Serrano-Ron, L., Perez-Garcia, P., Sanchez-Corrionero, A., Gude, I., Cabrera, J., Ip, P.-L., Birnbaum, K. D. and Moreno-Risueno, M. A. (2021). Reconstruction of lateral root formation through single-cell RNA sequencing reveals order of tissue initiation. *Mol. Plant* **14**, 1362-1378. doi:10.1016/j.molp.2021.05.028

Setty, M., Kiseliovas, V., Levine, J., Gayoso, A., Mazutis, L. and Pe'er, D. (2019). Characterization of cell fate probabilities in single-cell data with Palantir. *Nat. Biotechnol.* **37**, 451-460. doi:10.1038/s41587-019-0068-4

Shahan, R., Hsu, C.-W., Nolan, T. M., Cole, B. J., Taylor, I. W., Greenstreet, L., Zhang, S., Afanassiev, A., Vlot, A. H. C., Schiebinger, G. et al. (2022). A single-cell Arabidopsis root atlas reveals developmental trajectories in wild-type and cell identity mutants. *Dev. Cell*. doi:10.1016/j.devcel.2022.01.008

Shulse, C. N., Cole, B. J., Ciobanu, D., Lin, J., Yoshinaga, Y., Gouran, M., Turco, G. M., Zhu, Y., O'Malley, R. C., Brady, S. M. et al. (2019). High-throughput single-cell transcriptome profiling of plant cell types. *Cell Rep.* **27**, 2241-2247.e4. doi:10.1016/j.celrep.2019.04.054

Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W. M., Hao, Y., Stoeckius, M., Smibert, P. and Satija, R. (2019). Comprehensive integration of single-cell data. *Cell* **177**, 1888-1902.e21. doi:10.1016/j.cell.2019.05.031

Sunaga-Franze, D. Y., Muino, J. M., Braeuning, C., Xu, X., Zong, M., Smaczniak, C., Yan, W., Fischer, C., Vidal, R., Kliem, M. et al. (2021). Single-nucleus RNA sequencing of plant tissues using a nanowell-based system. *Plant J.* **108**, 859-869. doi:10.1111/tpj.15458

Tian, C., Du, Q., Xu, M., Du, F. and Jiao, Y. (2020). Single-nucleus RNA-seq resolves spatiotemporal developmental 1 trajectories in the tomato shoot apex. *bioRxiv*. doi:10.1101/2020.09.20.305029

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N. J., Livak, K. J., Mikkelsen, T. S. and Rinn, J. L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.* **32**, 381-386. doi:10.1038/nbt.2859

Wang, Y., Huan, Q., Li, K. and Qian, W. (2021). Single-cell transcriptome atlas of the leaf and root of rice seedlings. *J. Genet. Genomics* **48**, 881-898. doi:10.1016/j.jgg.2021.06.001

Wu, F.-H., Shen, S.-C., Lee, L.-Y., Lee, S.-H., Chan, M.-T. and Lin, C.-S. (2009). Tape-Arabidopsis sandwich - a simpler Arabidopsis protoplast isolation method. *Plant Methods* **5**, 16. doi:10.1186/1746-4811-5-16

Xia, K., Sun, H.-X., Li, J., Li, J., Zhao, Y., Chen, R., Liu, G., Chen, Z., Yin, R., Hao, S. et al. (2021). Single-cell Stereo-seq enables cell type-specific spatial transcriptome characterization in Arabidopsis leaves. *bioRxiv*. doi:10.1101/2021.10.20.465066

Yan, H., Song, Q., Lee, J., Schiefelbein, J. and Li, S. (2020). Identification of cell-type marker genes from plant single-cell RNA-seq data using machine learning. *bioRxiv*. doi:10.1101/2020.11.22.393165

Zhang, T.-Q., Xu, Z.-G., Shang, G.-D. and Wang, J.-W. (2019). A single-cell RNA sequencing profiles the developmental landscape of arabidopsis root. *Mol. Plant* **12**, 648-660. doi:10.1016/j.molp.2019.04.004

Zhang, T.-Q., Chen, Y. and Wang, J.-W. (2021a). A single-cell analysis of the Arabidopsis vegetative shoot apex. *Dev. Cell* **56**, 1056-1074.e8. doi:10.1016/j.devcel.2021.02.021

Zhang, T.-Q., Chen, Y., Liu, Y., Lin, W.-H. and Wang, J.-W. (2021b). Single-cell transcriptome atlas and chromatin accessibility landscape reveal differentiation trajectories in the rice root. *Nat. Commun.* **12**, 2053. doi:10.1038/s41467-021-22352-4