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Review article

Review: Challenges and perspectives in applying single nuclei RNA-seq technology in plant biology

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ABSTRACT

Plant single-cell RNA-seq technology quantifies the abundance of plant transcripts at a single-cell resolution. Deciphering the transcriptomes of each plant cell, their regulation during plant cell development, and their response to environmental stresses will support the functional study of genes, the establishment of precise transcriptional programs, the prediction of more accurate gene regulatory networks, and, in the long term, the design of *de novo* gene pathways to enhance selected crop traits. In this review, we will discuss the opportunities, challenges, and problems, and share tentative solutions associated with the generation and analysis of plant single-cell transcriptomes. We will discuss the benefit and limitations of using plant protoplasts vs. nuclei to conduct single-cell RNA-seq experiments on various plant species and organs, the functional annotation of plant cell types based on their transcriptomic profile, the characterization of the dynamic regulation of the plant genes during cell development or in response to environmental stress, the need to characterize and integrate additional layers of –omics datasets to capture new molecular modalities at the single-cell level and reveal their causalities, the deposition and access to single-cell datasets, and the accessibility of this technology to plant scientists.

1. Introduction: Why do we need to gain high-resolution transcriptomic knowledge?

The human population is expected to reach 9.7 billion people by 2050, an increase of 2 billion people compared to 7.7 billion people in 2019 (i.e., a predicted 25% increase in 30 years). To date, plant breeding has been very successful to deliver highly productive crop varieties with enhanced selected traits that benefit both human and animal consumption. To continue to feed a growing human population, crop yield must continue to increase. Besides, plant scientists also need to consider another major challenge when developing the next generation of crop species: the impact of climate change on crop production.

Plant breeding (i.e., Breeding 1.0–4.0 (Wallace et al., 2018)) has been very successful in supporting food production. As a perspective, Wallace et al., 2018 (Wallace et al., 2018) also raised in their review the concept of Breeding 5.0 where new sources of genetic diversity will be created and added to existing genotypes to engineer new functional pathways and new traits. Considering that each cell/cell type differentially uses its genomic information, there is a need to develop cell-type-specific engineering strategies to optimize the biology of each plant cell/cell type. The recent emergence of single-cell –omic technologies reveals the differential expression of genes from the same DNA molecule between cells and cell types and opens a door to a better understanding of gene function, gene regulation, and the prediction in gene networks. Considering that the biology of each cell/cell type is different, plant scientists will be able to use this new knowledge to personalize cell-type-specific genomic engineering strategies and to design *de novo* genes, with the ultimate goal to enhance the biology of each cell/cell type composing the plant and implement Breeding 5.0 strategies. For instance, the selection of cell-type-specific and/or stress-inducible promoter sequences will help control the expression of a transgene where and when relevant to the biology of the cell.

To date, our current understanding of plant transcriptomes is limited by the resolution of the information gained. Until recently, most transcriptomic information was collected from the entire organ, masking the unique use of the genomic information by each cell/cell type and the dynamic and differential regulation of the plant genes during development and in response to stresses. The recent implementation of single-cell/nucleus RNA-seq (sc/sNucRNA-seq) technology on various plant species and organs highlights the differential expression of genes between each cell and cell type composing the organ (Denyer et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019; Jean-Baptiste et al., 2019; Xie et al., 2020; Li et

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al., 2021a, 2021b; Liu et al., 2021; Wang et al., 2021; Xu et al., 2021; Satterlee et al., 2020; Ma et al., 2021; Lee et al., 2019; Liu et al., 2020; Lopez-Anido et al., 2021; Song et al., 2020; Kao et al., 2021; Zhang et al., 2021a; Shahan et al., 2022; Farmer et al., 2021) and suggests the broad application of these technologies in plant biology (Jha et al., 2021). Therefore, it is legitimate to question the future relevance of organ-based transcriptomes and their interpretation (e.g., functional characterization of plant genes, correlation analysis between various –omics datasets, prediction of gene regulatory networks (GRNs), design of biologically sounded genetic engineering strategies). For instance, gene co-expression should be revisited at the single cell-type level assuming that each cell/cell type is characterized by a unique epigenome, transcriptome, and proteome. Therefore. accessing different molecular modalities at the single-cell level will benefit the integration of various -omics datasets. In this review article, we discuss existing capabilities for plant biologists to understand plant cell biology at an unprecedented level of resolution, the current challenges associated with the use of these technologies, and the problems limiting our understanding of plant cell biology.

2. Which biological entity should be analyzed: cells or nuclei?

Accessing plant transcriptome at the single cell level requires the separation and then the isolation of undamaged biological entities (i.e., cell/protoplast or nucleus; Fig. 1). Any damage to a cell or nucleus would lead to the loss of single-cell information, low numbers of UMIs (Unique Molecular Identifier) and expressed genes per cell/nucleus, and the generation of noise in the transcriptomic datasets. While bioinformatics tools can help mitigate these problems, any scRNA-seq experiment should start with a careful selection of the biological entity to isolate (i.e., cell versus nucleus) followed by the optimization of the method used to isolate biological entities.

Drawbacks when using protoplasts are 1) their isolation which depends on the efficiency of enzymatic cocktails used to digest the cells wall; and 2) their fragility and the associated risk of bursting notably during the Gel bead-in EMulsions (a.k.a., GEMs) formation (Farmer et al., 2021; Long et al., 2021) or when applying the combinatory indexing approach (Sunaga-Franze et al., 2021; Picard et al., 2021). This is especially a concern when manipulating large plant protoplasts (e.g., up to 130 µm for hypocotyl-derived *Phaseolus vulgaris* protoplasts (Nanjareddy et al., 2016)). In contrast to protoplasts, nuclei isolation theoretically allows access to all the cell types composing the organ including those recalcitrant to cell degradation enzymes, leading to a better representation of the cell type diversity (see (Thibivilliers & Libault, 2021) for more information on this topic).

The cellular and nuclear transcriptomes are not equivalent. The cellular transcriptome is composed of nuclear and cytosolic polyadenylated transcripts. Considering the half-life of a transcript (i.e., up to 24 h (Narsai et al., 2007); Sorenson et al., 2018), the cellular transcriptome is an integration of gene activity over time. The nuclear transcriptome is less complex and composed of a smaller population of polyadenylated transcripts compared to the cellular transcriptome. The differential complexity between the cellular and nuclear transcriptome requires different sequencing depths to saturate a nuclear vs a cellular transcriptome. The nuclear transcriptome could be considered as a snapshot of the dynamic transcriptional activity of the genes providing a more dynamic picture of gene activity. Likely for these reasons, and upon optimization of the sNucRNA-seq technology, we found that the nuclear transcriptome better correlates with the profile of chromatin accessibility than the cellular transcriptome (Farmer et al., 2021).

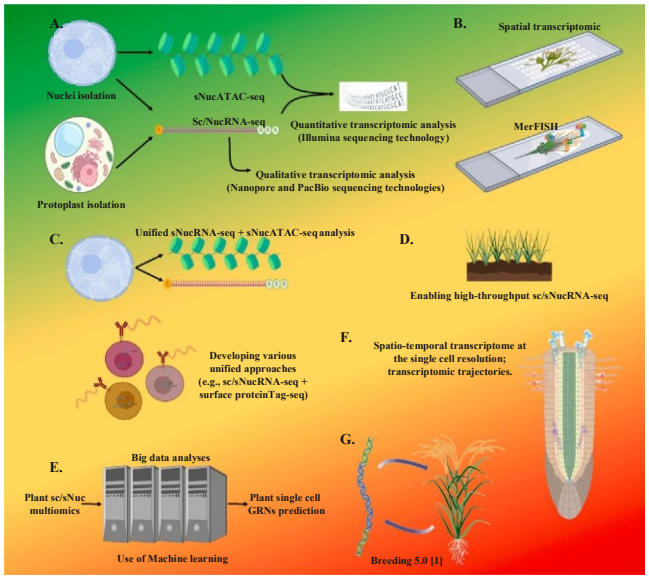

Fig. 1. Current advances and remaining challenges in plant single cell/nucleus omics biology. Using isolated nuclei or protoplasts from various plant species and organs, the patterns of gene expression and profiles of chromatin accessibility were characterized at the single cell level (A.). The emerging use of Spatial transcriptomic, MerFISH, and other spatially resolved transcriptomic approaches will support the annotation of plant cell types according to their transcriptional profiles (B.). More challenges need to be tackled. First, unified molecular information need to be collected to enhance our understanding of plant cell as biological systems (e.g., Unified "sNucRNA-seq + sNucATAC-seq" or "sc/sNucRNA-seq + surface proteinTag-seq" analyses) (C.). There is also a need to promote broader use of single-cell RNA-seq technology notably by reducing its cost. Developing high-throughput sc/sNucRNA-seq will support the broader use of these technologies (D.). Concomitantly to collecting data, bioinformatics workflows need to be developed to enable the prediction of gene regulatory networks (E.) and an understanding of the transcriptomic changes between cells and during their development (F.). Taking together, single-cell –omics technology has the potential to enhance our understanding of gene function and regulation and the implementation of better design de novo gene strategies to enable Breeding 5.0 (Wallace et al., 2018) (G.). The colors of the background reflect current advance in plant single cell biology (green) to its major challenge (red). "Created with BioRender.com.".

2.1. Getting the most from an sc/sNucRNA-seq library

The number of cells or nuclei to analyze has a profound influence on the outcome of single-cell transcriptome analyses and depends on the number of cell types composing the organ and their relative abundance. For instance, Chen et al. (2021) recently estimated the impact of the number of cells analyzed on the outcome of Arabidopsis root scRNA-seq analysis (Chen et al., 2021). They found that 20,000 cells led to the identification of the most significant components. Of course, this overall number of cells/nuclei analyzed is the result of the analysis of independent biological replicates. However, in some cases, the transcriptomic analysis of tissues and organs characterized by a lower cellular complexity would require a smaller number of cells to analyze. An example is a single-cell analysis of premeiotic and meiotic maize cells conducted on a few hundred cells (Nelms & Walbot, 2019). Online tools are publicly accessible to estimate the size of the population of cells/nuclei to analyze (https://satijalab.org/howmanycells/). In the end, the number of cells or nuclei selected for an sc/sNucRNA-seq transcriptomic analysis depends on the cellular complexity of the sample analyzed while the quality of transcriptomes would depend on the quality of the isolated cells and nuclei.

Sequencing depth would depend on 1) the transcriptional activity of the sample analyzed that varies between cells, cell types, and organs; 2) and the quality of the library preparation (e.g., protoplast bursting and RNA leakage would strongly decrease the complexity of the sequencing libraries).

2.2. Interpretation of sc/sNucRNA-seq transcriptomes

Generating high-quality sc/sNucRNA-seq datasets is a must. Their quality can only be evaluated only upon analysis. Quality criteria include the numbers of UMI identified (i.e., individual transcript), the number of cells analyzed, and the identification and removal of occurrences of doublets (i.e., droplets containing two or more cells) by applying for doublet detection bioinformatic programs (Adam Gayoso, 2022; Xi & Li, 2021)]. After pre-processing, commonly used computational packages, such as Seurat (Hao et al., 2021) and Monocle (Cao et al., 2019), are used for the analysis of sc/sNucRNA-seq libraries. These packages perform the normalization and the correction of the sequencing datasets to detect sequencing bias and noise, and to integrate independent biological replicates before applying dimensional reduction techniques to the high-dimensional sc/sNucRNA-seq data. These techniques include t-Distributed Stochastic Neighborhood Embedding (t-SNE) and Uniform Manifold Approximation and Projection (UMAP) and are employed to reduce the complexity and facilitate the visualization and downstream analysis.

2.3. Tell me what your RNAs are and I will tell you who you are: the biological interpretation of single cell-resolution transcriptomics

Model species (e.g., *Arabidopsis thaliana*, *Medicago truncatula*, *Oryza sativa*, *Zea mays*) benefit from resources that support the biological interpretation of sc/sNucRNA-seq datasets such as the identification of cell-type marker genes. Therefore, upon characterization of the transcriptomic profiles of the different cells and cell types composing an organ from a model plant species, the functional annotation of the cell clusters according to the transcriptomic profiles of these marker genes can be conducted. Such an approach strongly facilitated the analysis of the transcriptome of Arabidopsis root cells based on the large pool of information available (Denyer et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019; Jean-Baptiste et al., 2019; Shahan et al., 2022; Farmer et al., 2021).

Non-model species, including numerous crop species, do not benefit from the functional characterization of a large number of cell-type marker genes in comparison to model species. This is a challenge when annotating sc/sNucRNA-seq clusters. Different strategies could be implemented to overcome this limitation. *In silico*, putative cell-type marker genes from non-model species can be assessed assuming the conservation of the transcriptional pattern between plant orthologs (e.
g., see (Kryuchkova-Mostacci & Robinson-Rechavi, 2016) for a comprehensive analysis of the conservation of gene expression between animal orthologs). Experimentally, in situ hybridization and the transcriptional fusion between the promoter of cell-type marker genes and a reporter have been extensively used to analyze the transcriptional activity of selected genes *in planta* (see (Moses & Pachter, 2022) for a complete list of technologies used to reveal spatially localized quantification of messenger RNA). The low throughput and labor-intensive nature of these methods are major limitations when considering the annotation of tens of different cell types. Besides, the analysis of the activity of transcriptional fusion *in planta* presupposes the capability to transform plants.

To overcome these limitations, spatial transcriptomic technologies emerge as a valid approach to complement single cell/nucleus RNA-seq as these technologies allow the visualization of the localization of individual transcripts in the context of the cellular architecture of the organ. Two strategies exist 1) the *in planta* tagging of transcripts with fluorescent probes before their visualization with confocal microscopy [e.g., Molecular Cartography (Resolve Biosciences; MERSCOPE (Vizgen); Phyotmap (Nobori et al., 2022)]; 2) the release from the cells of the polyadenylated transcripts and their attachment to printed oligonucleotides leading to the unique barcoding of cDNAs according to their location on the capture area and the mapping of the sequenced cDNA in the context of the morphology of the tissue [e.g., Visum Spatial Transcriptomics (10x Genomics)]. Spatial transcriptomic technologies have several advantages: 1) they can be applied to non-transgenic plant material; 2) they currently support the analysis of the expression of hundreds to thousands of genes at the same time [see (Moffitt et al., 2022) for review; Fig. 1]. Animal scientists have already used spatial transcriptomic technologies to reveal single-cell resolution transcriptomic datasets in the context of the organization of the tissue (Ståhl et al., 2016; Franses et al., 2022; Hu et al., 2022), while in plant biology, the use of these technologies is currently emerging (Giacomello et al., 2017). We can expect to see an increase in the use of spatial transcriptomics technologies to support the annotation of plant cells according to their transcriptomic profiles in the near future.

The annotation of the different cell/nucleus clusters based on their transcriptomic profile offers an opportunity to compare the transcriptome of specific cell types between plant organs and plant species. To implement an inter-organ comparative analysis, single-cell resolution transcriptome atlases must be generated (i.e. similar to the *Tabula Sapiens* atlas for humans (Jones et al., 2022)). Comparative analysis between plant species will benefit from the expertise of evolutionary biologists to reveal the sets of homeologs, paralogs, and orthologs. As a first effort to enable cross-species comparison of single-cell transcriptomic datasets, Zhang et al. (2021) conducted a comparative analysis of the single cell transcriptomes of Arabidopsis and Rice focusing on the one-to-one orthologs. Their analysis revealed that highly specialized cell types such as xylem, phloem, and root epidermal cells shared the most conserved transcriptomic profiles (Zhang et al., 2021b). We assume that a similar approach will require the development of unique strategies when comparing the transcriptional activity of plant genes from species with different levels of ploidy where more than one-to-one orthologs can be identified.

2.4. Use of single-cell –omics to develop new knowledge in plant biology

2.4.1. Deciphering the dynamic regulation of the transcriptomic programs controlling plant cell development, cell states, and response to environmental stress

Previous studies reported cell-type-specific transcriptomic responses to environmental stresses and hormones (e.g., sugar, drought, and salt stress conditions; in response to ethylene (Shulse et al., 2019); Wang et al., 2021; Liu et al., 2022a; Zeng et al., 2021; Liu et al., 2022b). These findings suggest that single-cell resolution transcriptomic analyses have the potential to detect finely tuned transcriptomic changes between cell types, and cell-type-specific transcriptomic responses to environmental stresses. The differential clustering of the cells in response to stress and the identification of cell-type-specific differentially expressed genes will enhance our understanding of plant cell response to abiotic and biotic stress, and plant development. Additionally, single-cell transcriptomics offers an opportunity to explore dynamic changes in cellular states or programs through the analysis of selected mutants (Shahan et al., 2022) by implementing developmental trajectory analysis (Van den Berge et al., 2020). Such approaches have been implemented to reveal the different cell states of the Arabidopsis root and leaf (Lopez-Anido et al., 2021; Shahan et al., 2022).

2.4.2. Single-cell resolution multiomic approaches

While sc/sNucRNA-seq technology provides a new understanding of the differential use of the genomic DNA between cells and cell types, an additional layer of molecular information will provide a deeper understanding of plant cell biology. Multiomic approaches have become largely used by human and mammalian biologists, as it provides access to the transcriptome and profile of chromatin accessibility (Orchard et al., 2021; Allaway et al., 2021; Gegenhuber et al., 2022), or the transcriptome and the proteome of single cells (Stoeckius et al., 2017; Mair et al., 2020) (Fig. 1). The latter was facilitated by commercially available protein surface antibodies that we are currently lacking in plant biology. Therefore, we will not discuss the implementation of the transcriptome/proteome approach on plant cells in this review. The analysis of gene expression (Farmer et al., 2021; Long et al., 2021; Sunaga-Franze et al., 2021; M´eteignier, 2022; Neumann et al., 2022) and profile of chromatin accessibility (Farmer et al., 2021; Marand et al., 2021; Dorrity et al., 2021) at the single cell level has been independently conducted using nuclei isolated from different organs and plant species. However, the

implementation of a "real" multiomic approach (i.e., the simultaneous capture of gene expression and profile of chromatin accessibility from the same biological entity to unify different molecular modalities) remains challenging based on the requirements of this approach. A unified sNucRNA-seq + sNucATAC-seq analysis requires the permeabilization of the nuclear membrane to promote the penetration of the Tn5 enzyme inside the nuclei for the tagmentation of the genomic DNA but without inducing RNA leakage. As a reflection of these challenges, Orchard et al., (2021) generated independent sNucRNA-seq, sNucATAC-seq, and multiome libraries and noticed losses of ~ 10% and ~46% of the number of transcripts and ATAC fragments detected when comparing single versus multiomic approaches, respectively (Orchard et al., 2021). New methods will be needed to mitigate these problems because the concomitant analysis of gene expression and profile of chromatin accessibility has the potential to directly identify cell type-specific cis-regulatory elements (CREs), reveal the influence of chromatin accessibility on gene expression, and, when conducted between species and organs, to expose the level of conservation of these mechanisms. Accessing such knowledge will support the design of *de novo* genes and the implementation of Breeding 5.0 strategies at the single cell/cell-type resolution (Fig. 1).

2.4.3. Impact of single-cell study to analysis plant biotic interactions

Sc/sNucRNA-seq technology has the potential to answer many of the major questions in molecular plant-microbe interactions (Harris et al., 2020) because it will reveal the heterogeneity of the responses of the host cells according to their infection stage. Accessing the transcriptome of cells at different stages in their recognition, interaction, and then infection by bacteria, fungi, viruses, nematodes will provide a very dynamic picture of these biological interactions. The implementation of transcriptomic trajectory analysis to create, *in silico*, the pseudo-time line of an infection process would have a strong impact on our understanding of plant biotic interactions.

When considering the interaction between plants and other eukaryotic organisms (i.e., fungi, nematodes), it might be possible that a sNucRNA-seq approach might capture the transcriptional response of the plant host cells and the cells of the pathogen. Such information will provide a deeper understanding of the programs concomitantly used by the plant and its pathogen during infection.

There are currently two limitations to the use of sc/sNucRNA-seq technology to study plant biotic interactions. The first one is associated with the limited number of events of infection occurring on the plant. The implementation of strategies to tag infected cells (e.g., the use of a pathogen-inducible promoter cloned upstream to a reporter) could be an attractive strategy to focus the analysis on the infected cells. The second limitation is the loss of physical interaction between the plant cells and the pathogen upon nuclei insolation. To better understand the intimate molecular responses between those interacting cells, sc/ sNucRNA-seq technology should be combined with spatial transcriptomic technology that will preserve the cellular and tissular organization of the plant host and its pathogen (Saarenpa¨a et al., 2022¨).

3. Remaining challenges

Cells or nuclei? The use of plant protoplasts to conduct single-cell transcriptomic analysis will likely remain difficult for two reasons: 1) the optimization of the method to efficiently digest the cell wall will differ based on the age and nature of the organ and between plant species (Sarkar et al., 2009); 2) the large size of plant protoplasts would not be compatible with the diameter of the optical fiber of current microfluidic devices. Based on their ease to be isolated, plant nuclei should, in theory, allow the characterization of the transcriptome of each cell from any plant organ and plant species. Taking into consideration the possibility to access different molecular modalities to understand gene expression and its regulation (e.g., gene expression levels and profiles of chromatin accessibility), we foresee that the broad application of single-cell technology in plant biology will rely on the use of isolated nuclei as an input. *3.1. Capturing plant bacterial/viral interactions*

Current sc/sNucRNA-seq technology starts with the reverse transcription of the polyadenylated transcripts. This first step prevents the capture of most of the bacterial, viral, and small RNA transcriptomes. Alternative approaches must be developed to capture prokaryotic and the entire eukaryotic transcriptomes at the single-cell level.

3.2. The complexity of plant genomes

Another distinguishing feature between plants and animals is the higher frequency of polyploidy events of the plant genomes that result in larger genome sizes (Soltis et al., 2015). Therefore, structural and genomic differences could lead to plant-specific challenges in plant single-cell transcriptome analysis, and downstream analysis between plant species, organs, tissues, or plant genome sizes.

3.3. Deposition and access to single-cell datasets

The format of raw sequencing files (i.e., FASTQ files), files that contain the number of UMIs, information about the number of cells/ nuclei per cluster, number of samples, etc. (i.e.,.rds files), and metadata associated with an experiment will need to be standardized. The Plant Cell Atlas framework ($author1$ et al., 2021) will play a critical role in promoting good practices in sharing sc/sNucRNA-seq datasets and resources, and in bridging various repository hubs and existing online single-cell genomic/transcriptomic browsers (Xu et al., 2022; Ma et al., 2020). We expect these resources to become more complex upon the integration of various single-cell molecular modalities and their integration to generate single-cell-type resolution GRNs.

4. Conclusion: the future of sc/sNucRNA-seq technology in plant biology

The broad access to plant sc/sNucRNA-seq technology suffers from four different and independent bottlenecks: cost (see https://satijalab.org/costpercell/ for an estimation), access to facilities, expertise in sample preparation, and bioinformatics capability for data analysis. Considering that the manipulation of a plant sample is significantly different from an animal sample, it could be assumed that core facilities with sc/sNucRNA-seq capabilities would need to optimize their methodology before conducting sc/sNucRNA-seq experiments on plant samples. Additional expertise will be needed to implement spatial transcriptomic analysis to support the interpretation of sc/sNucRNA-seq datasets. While spatial transcriptomic technologies might replace sc/ sNucRNA-seq technology to quantify plant gene activities in specific organs, we believe that spatial transcriptomic technologies will expand into two main directions: the capture of various molecular datasets from the same cells (e.g., transcriptomic, epigenomic, proteomic, etc.); and their broad application on a large number of samples upon implementation of multiplexing strategies. The latter will enable the application of sc/sNucRNA-seq plant populations to better capture the impact of genes on unique phenotypes and the development of Breeding 5.0 strategies (Wallace et al., 2018). Therefore, in the future, researcher centers with expertise in flow cytometry, microscopy, single-cell omics, and bioinformatics would have an opportunity to leverage plant sc/sNuc -omic technologies.

The future of plant sc/sNucRNA-seq technology is not limited to the quantification of gene expression at the single-cell level. Qualitative information could also be gained such as the differential splicing of transcripts at the single cell level. To access this information at the single cell level, researchers can rely on the constant development of long-read sequencing platforms (e.g., Oxford Nanopore and Pacific Biosciences) allowing the sequencing of entire cDNAs and their associated cell/nucleus and UMI tags (Fig. 1). Such an approach has been used on human cells to reveal the role of splicing variants in neurological disease (Habib et al., 2016; Hardwick et al., 2022) and on Arabidopsis root cells with success (Long et al., 2021). In this latter publication, Long et al. (2021) not only demonstrated that the nuclear transcriptome is sufficient for clustering nuclei according to their cell type but also highlighted the benefit of accessing long read sequences to better define

cell clusters by adding another layer of molecular information (i.e., alternative splicing and polyadenylation of the transcript) (Long et al., 2021). Alternative approaches exist to access full cDNA reads. For instance, the generation of fragmented and poly(A)-tailed RNA was implemented to access the sequence of full cDNA reads as well as non-polyadenylated transcripts (Salmen et al., 2022). The addition of different types of molecular information at the single cell level (i.e., epigenomic marks, chromatin remodeling, gene expression, differential splicing, RNA structure, a pool of non-coding RNAs, protein and metabolite abundances, etc.) will continue to enhance our understanding of plant cell biology as individual and interacting systems. To reach such knowledge, unified multiomic must be implemented where different types of molecular information can be captured from the same cell at the same time. The integration and interpretation of this information in the context of plant development, cellular differentiation, and plant cell response to environmental stress will likely require new mathematical and bioinformatical models supported by the use of machine learning (Fig. 1).

Understanding the regulatory mechanisms controlling gene expression is a major goal in plant biology. During the past two decades, whole- organ transcriptomic studies through the use of microarrays and RNA- seq technologies have advanced our understanding of these mechanisms including the inference of GRNs. However, it is important to recognize the limitations of these studies including the access to many independent transcriptomic datasets to generate accurate GRNs, and the noise of the transcriptomic data generated from cellularly heterogeneous plant samples. Single-cell transcriptomics has the potential to support the prediction of shared and cell-type-specific GRNs (Ferrari et al., 2022). Plant scientists should consider the promissory advances in the inference of GRNs at the single-cell level in animals and yeast using novel correlation metrics (Iacono et al., 2019; Jackson et al., 2020). Other challenges to implementing GRN prediction at the single-cell resolution are associated with data management, the computing requirements to obtain more accurate GRNs, variation of sequencing depth between cells, the sparsity of the data (Kharchenko et al., 2014), and batch effect (Li et al., 2020). In this sense, few methods have been used to infer GRNs from single-cell data including BEELINE which is a compendium of GRN programs to evaluate the accuracy, robustness, and efficiency of GRNs (Pratapa et al., 2020). The development of innovative bioinformatic tools and the use of deep (Yuan & Bar-Joseph, 2019) or hybrid deep machine learning (Zhao et al., 2022) will assist plant biologists in predicting cell-type-specific GRNs. We expect that enhanced cell-type-specific GRNs will benefit from the integration of unified multiomic analyses including the spatial and temporal transcriptional patterns of the genes (de Luis Balaguer et al., 2017), the profiles of chromatin accessibility and long-range chromatin interactions, and the pool of microRNAs (Qian & S.-s.C. Huang, 2020), etc.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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