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Decoding Plant Cellular Complexity Through Single-Cell Omics Approaches

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Abstract: Cells form the foundational units of life, yet individual cells within the same organism often differ substantially in their molecular makeup and functional behavior. Understanding these cell-to-cell variations is essential for deciphering complex biological processes, particularly in plants where single-cell studies are still emerging. Single-cell omics technologies enable the characterization of cellular diversity by examining genomes, epigenomes, transcriptomes, proteomes, and metabolomes at the resolution of individual cells. These approaches reveal functional heterogeneity and lineage relationships that cannot be captured through bulk tissue analysis. Recent progress in cell isolation, microfluidics, amplification chemistry, and high-throughput sequencing has transformed the study of unicellular profiles across diverse organisms. This review summarizes the major single-cell omics platforms, discusses plant-specific challenges, and outlines how these technologies contribute to understanding development, stress biology, and cellular specialization. Together, these tools offer an unprecedented window into plant cellular complexity and hold promise for advancing crop improvement strategies.

Keywords: *Microfluidic, Enzymolysis, Micromanipulation, Fluorescent Activated Cell Sorting (FACS), Pollen Typing.*

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I. INTRODUCTION

Cells represent the fundamental biological units responsible for growth, metabolism, communication, and structural organization in all living organisms. Although cells within the same tissue share a common genetic blueprint, they frequently exhibit striking differences in morphology, gene expression, and biochemical activity. These variations shape how tissues function and how organisms respond to developmental and environmental cues. Traditional bulk-level analyses average signals from thousands of cells, masking this intrinsic heterogeneity and preventing the detection of rare or transient cellular states. The emergence of single-cell omics has begun to overcome these limitations by enabling researchers to study biological systems one cell at a time (Shapiro et al., 2013; Mincarelli et al., 2018).

Single-cell omics integrates multiple high-resolution molecular approaches—including genomics, transcriptomics, proteomics, and metabolomics—to define the identity and state of individual cells. Each of these layers captures a different aspect of cellular function: the genome reflects inherited information, the transcriptome captures dynamic gene activity, the proteome reveals functional effectors, and the metabolome represents immediate biochemical outcomes (Efroni & Birnbaum, 2016; Zenobi, 2013). By combining these datasets, researchers are now able to reconstruct lineage relationships, track developmental trajectories, and uncover regulatory networks that would otherwise remain hidden in population-level studies.

Plant biology presents both unique challenges and exciting opportunities for single-cell research. The rigid cell wall complicates tissue dissociation and often necessitates enzymatic digestion, which can stress cells and interfere with molecular readouts (Efroni et al., 2015; Jia et al., 2016). Moreover, plant genomes vary widely in size and composition, and plants produce diverse secondary metabolites that may inhibit molecular assays (Gregory, 2005). Despite these difficulties, rapid technological advances—including improved protoplast isolation, microfluidic sorting, laser-based capture methods, and high-throughput RNA sequencing—have expanded the feasibility of profiling individual plant cells (Gawad et al., 2016; Whitesides, 2006).

Single-cell studies have already reshaped understanding in several areas of plant science. High-resolution transcriptomic analyses have revealed the molecular identities of specialized cells such as root hairs, trichomes, and cotton fibers, uncovering regulatory signatures that were not apparent in bulk tissue profiles (Libault et al., 2010;

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Betancur et al., 2010; Haigler et al., 2009). Similarly, single-cell approaches have been instrumental in studying regeneration processes, showing that damaged Arabidopsis roots can quickly re-establish stem cell patterns that resemble embryogenesis (Efroni et al., 2016). These findings highlight the developmental plasticity of plant cells and illustrate how single-cell technologies can illuminate cell fate transitions.

In stress biology, single-cell transcriptomics is helping clarify hormone interactions and signaling pathways that govern responses to environmental challenges. Bulk analyses often provide conflicting interpretations of these pathways, particularly regarding ethylene, jasmonate, and abscisic acid crosstalk during abiotic stress (Ghassemian et al., 2000; Cheng et al., 2009; Song et al., 2014). By measuring gene expression directly within individual cells, researchers can now distinguish hormone responses specific to particular cell types or developmental stages (Coolen et al., 2016; Breakspear et al., 2014). This level of detail is essential for accurately modeling plant stress adaptation.

Another promising direction is the application of single-cell genomics in plant reproductive biology. Sequencing individual pollen grains allows direct measurement of recombination, haplotypes, and allele segregation without the need for long generational cycles, making it an efficient alternative for quantitative trait locus (QTL) mapping and crop breeding (Chen et al., 2008; Dreissig et al., 2017). Such approaches demonstrate how single-cell technologies can accelerate the discovery of genes linked to important agronomic traits.

As single-cell platforms continue to improve in throughput, sensitivity, and cost-efficiency, they are expected to become routine tools in plant systems biology. The integration of genomic, epigenomic, transcriptomic, proteomic, and metabolomic datasets at single-cell resolution will enable comprehensive reconstruction of cellular networks, ultimately advancing efforts in crop improvement, developmental biology, and stress resilience research (Regev et al., 2017; Lin et al., 2017). Although challenges remain in cell preparation, amplification chemistry, and data interpretation, the rapid progress of single-cell omics promises to reshape both basic and applied plant science in profound ways.

II. SINGLE CELL ISOLATION METHODS IN PLANTS

Isolating individual plant cells is a crucial first step for any single-cell omics workflow, yet it remains one of the most technically challenging aspects of plant single-cell biology. Unlike animal cells, which can often be separated through gentle mechanical dissociation, plant cells are surrounded by a rigid and chemically complex cell wall that restricts physical separation. This wall, composed largely of cellulose, hemicellulose, pectins, and lignin, provides structural support but significantly limits access to the internal molecular components required for downstream omics analyses (Fang & Spector, 2005; Birnbaum, 2016). As a result, selecting an appropriate isolation method is essential to preserve cellular integrity and avoid introducing artifacts into molecular measurements.

The most widely used strategy for obtaining single plant cells is protoplasting, which involves enzymatic digestion of the cell wall using cellulases and pectinases. When optimized carefully, protoplasting can yield large numbers of viable, wall-less cells suitable for transcriptomic, genomic, and proteomic studies. This approach has been successfully applied in various species, enabling high-resolution analysis of root tissues, developing leaves, and reproductive structures (Long et al., 2014; Denyer et al., 2019; Satterlee et al., 2020). However, protoplasting is not without limitations. The enzymatic treatment itself can trigger stress responses that alter gene expression, complicating the interpretation of transcriptome data (Del Olmo et al., 2022; Lai et al., 2021). Additionally, certain cell types resist digestion, resulting in biased representations of the tissue.

To overcome biases associated with enzymatic digestion, alternative approaches such as single-nucleus isolation have gained prominence. Instead of targeting entire cells, this method releases nuclei from tissues using gentle mechanical disruption followed by flow sorting. Isolated nuclei provide stable samples for downstream assays like single-nucleus RNA sequencing, which has proven especially valuable for analyzing mature or lignified plant tissues where protoplast generation is inefficient (Farmer et al., 2021). Although nuclear RNA differs from total cellular RNA, recent studies show that nuclei capture sufficient transcriptional information to reconstruct cell-type identities and developmental trajectories (Sunaga-Franze et al., 2022). The compatibility of nuclear isolation with frozen material further expands the range of tissues suitable for single-cell profiling (Schon et al., 2021).

Physical microdissection techniques also contribute to single-cell isolation, particularly when investigating rare or spatially localized cell types. Laser Capture Microdissection (LCM) allows researchers to precisely excise individual cells or small groups of cells from tissue sections under microscopic guidance. This method preserves spatial information and avoids enzymatic exposure, making it valuable for transcriptomic and epigenomic analyses in sensitive developmental stages (Nelson et al., 2006; O'Malley et al., 2016). However, the throughput of LCM is low compared to modern droplet-based platforms, and the extracted material may be limited.

As single-cell technologies evolve, microfluidic innovations are enhancing the efficiency and gentleness of cell isolation. Droplet-based systems, widely used in animal studies, can be adapted for plant protoplasts and nuclei once an appropriate suspension is prepared. These systems encapsulate individual cells into tiny droplets containing barcoded beads, allowing thousands of cells to be processed simultaneously (Macosko et al., 2015; Klein et al., 2015). Although droplet microfluidics does not solve the challenge of cell wall digestion, it dramatically improves scalability after cell or nuclear suspensions have been generated.

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Choosing the appropriate isolation method depends heavily on tissue type, experimental goals, and downstream analyses. Protoplasts provide intact cellular contents but may induce stress; nuclei avoid digestion artifacts but lack cytoplasmic information; microdissection preserves spatial context at the cost of throughput. As protocols continue to improve, combining multiple isolation strategies may provide the most accurate representation of plant cellular diversity. The refinement of these approaches is already enabling researchers to interrogate complex plant tissues at single-cell resolution, establishing a foundation for deeper insights into plant development, stress adaptation, and cellular specialization.

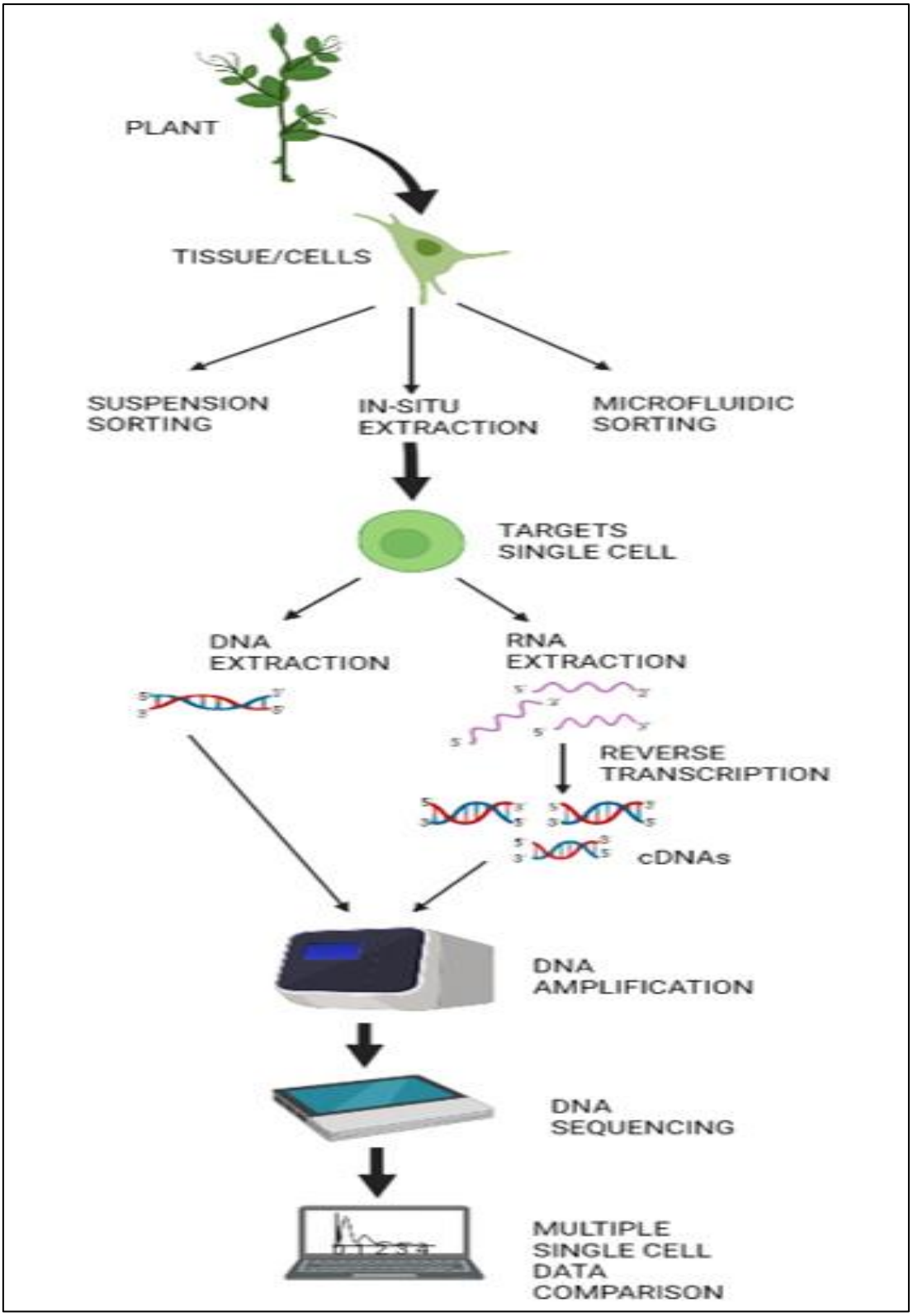

Fig 1 Workflow of Plant Single-Cell Omics, from Cell Isolation to Sequencing and Data Analysis.

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Table 1 Comparison of Various Single-Cell Isolation Techniques

Approach	Isolation techniques	Accuracy	Cell material requirement	Challenges	References
Suspension	Serial dilution	Very low accuracy	High	These techniques are very time consuming, contain low accuracy and requires large number of cells due to high mis identification rate and that may affect the yield of cell sub population and the cells may damage.	Ham,1965; Shapiro et al.,2013; Scangrude et al.,1988
	Micromanipulation	Moderate accuracy	Low		
	Fluorescence-activated cell sorting	High accuracy	High		
In-situ	Laser microdissection (LMD)	Moderate	High	The cells can be sliced accidentally, the cell nuclei can be damaged by UV rays and contamination may occur from neighbouring cells.	Emnert-Buck et al.,1996
	Laser microdissection and pressure catapulting (LMPC)	Moderate	High		
	Laser captured micro dissection (LCM)	Moderate	High		
Microfluidics	microfluidics	High	Moderate to high	This technique costs very high and need all uniform cell sizes.	Whitesides, 2006

III. SINGLE CELL GENOMICS IN PLANTS

Genomic information is generally stable across most cells within a multicellular organism, yet subtle variations can arise through somatic mutations, chromosomal rearrangements, or localized genomic alterations during development. These rare events are often obscured when DNA is extracted from bulk tissues, which average signals from many cells. Single-cell genomics makes it possible to examine genomic features at cellular resolution, enabling the detection of mosaicism, lineage-specific mutations, and structural variants that would otherwise remain undetected (Gawad et al., 2016; Vitak et al., 2017). For plants, where developmental plasticity and long lifespans can increase opportunities for somatic variation, the ability to sequence individual cells has significant implications for understanding genome dynamics and trait inheritance.

Despite its potential, single-cell genomic analysis in plants faces major technical hurdles. A single cell contains only picogram quantities of DNA, far below what is required for standard next-generation sequencing. To generate sufficient material, researchers rely heavily on whole-genome amplification (WGA). However, WGA can introduce amplification noise, allelic dropout, and uneven genomic coverage, complicating efforts to accurately interpret single-cell data (Hou et al., 2012). The complexity of plant genomes—including their large size, polyploidy, and repetitive elements—exacerbates these challenges. As a result, choosing an appropriate WGA strategy is a critical step in plant single-cell genomics.

Early WGA techniques, such as primer extension preamplification (PEP-PCR), degenerate oligonucleotide PCR, and linker-adapter PCR, provided initial proof that DNA from a single cell could be amplified (Zhang et al., 1992; Dean et al., 2002). However, these methods yielded highly variable coverage and introduced strong biases, limiting their suitability for high-resolution genomic applications. Multiple Displacement Amplification (MDA) marked a major improvement by using the high-fidelity phi29 DNA polymerase, which can generate long amplification products and achieve significantly better coverage of the genome (Spits et al., 2006; Zong et al., 2012). MDA has become one of the most widely applied WGA methods in plant studies, although it still suffers from coverage unevenness and difficulty detecting allelic variants with precision.

To further improve uniformity, researchers developed Multiple Annealing and Looping-Based Amplification Cycles (MALBAC), which combines quasi-linear and exponential amplification steps. MALBAC reduces allelic dropout and provides more even coverage across the genome compared to MDA, making it well suited for identifying copy number variations (Zhou et al., 2018). Nevertheless, MALBAC can introduce more false-positive single-nucleotide variants than desired, and thus is often used in combination with other validation approaches. Continued refinements in microfluidics have produced methods such as MIDAS, which partition genomic material into nanoliter-scale reaction chambers to improve amplification uniformity and reduce technical noise (Lasken & Stockwell, 2007).

Single-cell genomics is increasingly finding applications in plant reproductive and developmental research. One particularly powerful approach is the sequencing of individual pollen nuclei, which enables direct assessment of recombination patterns, haplotypes, and allelic segregation in a single generation (Dreissig et al., 2017; Morris et al., 2020). This strategy bypasses the need for constructing large segregating populations and offers a rapid means of identifying genomic regions linked to agronomic traits. Similarly, sequencing nuclei from plant meristems or developing organs provides insights into somatic mutation

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rates, genome stability, and lineage specification during growth (Ryu et al., 2019).

As sequencing technologies become more sensitive and amplification chemistries continue to improve, single-cell genomics is poised to become a mainstream tool in plant biology. The combination of powerful computational frameworks with more accurate WGA methods will allow researchers to map genomic variation at unprecedented resolution. For crop species, this holds great promise for accelerating breeding, identifying structural variants linked to yield or stress tolerance, and understanding how genetic diversity is generated and maintained within individual plants across their lifespans. Although technical constraints remain, the expanding toolkit for single-cell genomics is rapidly transforming our ability to explore the plant genome one cell at a time.

IV. SINGLE CELL TRANSCRIPTOMICS IN PLANTS

The transcriptome of a cell reflects its dynamic physiological state, capturing which genes are being actively expressed and how those expression levels change in response to developmental and environmental cues. Because individual cells within the same tissue can differ dramatically in their transcriptional activity, bulk RNA-sequencing masks important variation by averaging signals from many cell types. Single-cell transcriptomics, especially single-cell RNA sequencing (scRNA-seq), addresses this limitation by allowing gene expression to be measured in thousands of individual cells simultaneously, revealing patterns that would otherwise remain hidden (Tang et al., 2009; Macosko et al., 2015).

A central challenge in single-cell transcriptomics is the extremely small amount of RNA present in a single plant cell, which typically yields only femtograms of mRNA. To generate enough material for sequencing, transcripts must first be converted to complementary DNA (cDNA) and then amplified using whole transcriptome amplification (WTA). Early methods relied heavily on PCR amplification and often produced incomplete cDNA libraries, introducing bias toward the 3′ ends of transcripts (Tang et al., 2009). These limitations prompted the development of more sophisticated techniques such as SMART-seq, which employs a template-switching mechanism to capture full-length transcripts and significantly improves coverage across gene bodies (Ramsköld et al., 2012). The enhanced sensitivity and uniformity of SMART-seq2 later made it one of the most widely used protocols for studying plant protoplasts and nuclei (Picelli et al., 2014).

To further reduce amplification bias, researchers introduced in vitro transcription (IVT)–based approaches such as CEL-seq and CEL-seq2, which rely on linear rather than exponential amplification. These methods incorporate unique molecular identifiers (UMIs) that tag individual transcripts before amplification, enabling more accurate quantification by distinguishing true biological variation from technical noise (Hashimshony et al., 2016). Although IVT-based methods typically capture the 3′ ends of transcripts, their reduced bias and compatibility with high-throughput workflows have made them valuable tools for profiling complex plant tissues.

The most transformative advances in single-cell transcriptomics have come from droplet microfluidics, which dramatically increases throughput and reduces cost. Systems such as Drop-seq, inDrop, and the 10x Genomics Chromium platform encapsulate single cells in nanoliter droplets together with barcoded beads, enabling tens of thousands of cells to be profiled in a single experiment (Klein et al., 2015; Macosko et al., 2015). These methods are now widely applied in plant studies, particularly for tissues where robust protoplast isolation protocols exist, such as roots and young leaves (Shulse et al., 2019; Denyer et al., 2019). The scalability of droplet systems has allowed researchers to discover new cell types, reconstruct developmental pathways, and map transcriptional responses to environmental signals with unprecedented resolution.

For tissues that are difficult to digest into protoplasts—such as lignified stems, reproductive organs, or mature leaves—single-nucleus RNA sequencing (snRNA-seq) provides a powerful alternative. This method isolates nuclei rather than whole cells and therefore avoids stress responses associated with enzymatic digestion. Although nuclear RNA captures only a subset of the whole transcriptome, snRNA-seq has been shown to faithfully recover cell identities, developmental gradients, and stress-responsive transcriptional modules in diverse plant species (Farmer et al., 2021; Sunaga-Franze et al., 2022). The ability to use frozen tissues further expands its applicability to crops and field-grown plants.

Single-cell transcriptomics has already reshaped plant developmental biology. High-resolution expression maps have revealed molecular signatures of root meristem organization, vascular differentiation, and stomatal patterning (Jean-Baptiste et al., 2019; Shahan et al., 2022). In stress-response research, scRNA-seq has clarified hormone signaling pathways and identified cell-type–specific responses to pathogens, drought, and nutrient limitations—patterns that were ambiguous in bulk data (Ryu et al., 2019; Shaw et al., 2021). These insights highlight the power of single-cell approaches to disentangle the cellular complexity of plant tissues.

As sequencing chemistries, microfluidic platforms, and computational pipelines continue to improve, single-cell transcriptomics will play an increasingly central role in plant systems biology. The integration of scRNA-seq with spatial transcriptomics, epigenomic profiling, and proteomic measurements promises to produce multi-layered atlases of plant development and stress adaptation. Such integrative datasets will ultimately support crop improvement by identifying key regulators of cell identity and environmental resilience.

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Table 2 Different Approaches Practiced for Single Cell Omics

Techniques	Platform	Number of cells	Description of the methods	Applications	Typical number of sequencing reads per cell	References
Smart-seq/smart-seq2	Microwell plate/ tube/ fluidigm C1 platform	100s - 1000s	It is a PCR based full length transcript amplification, templates can be switched and can be applied to cells or nuclei	Alternative splicing can be analysed by this process, the number of transcripts can be counted and allelic expression may be studied	500000-4000000	Picelli et al.,2013 Picelli et al.,2014
CEL-seq/CEL-seq2	Microwell plate/tube	100s-1000s	3 times transcript amplification method and this is based in-vitro	Numerical analysis of transcripts	100000-1000000	Hashimsoni et al.,2012 Hashimsoni et al.,2016
STRT	Microwell plate / tube	100s-1000s	The template can be switched and it is a PCR based full length transcript amplification, followed by 5‘selection	Numerical analysis of transcripts	100000-1000000	Islam et al.,2011; Hochgerner et al.,2017
Sci-RNA	Combinatorial indexing	1000s-10000s	Combinatorial indexing technique is a technique in which during sauce strand synthesis and during PCR of 3‘sequencing tag the transcripts are first indexed	Numerical analysis of transcripts	20000-200000	Cao et al.,2017
Droplet-based approaches	Microfluidic platform: Drop-seq InDrop Commercial platforms: 10x genomics Chromium Dolomite Nadia	1000s-10000s	Individual droplets are formed when the cells are partitioned and during reverse transcription cDNA molecules are uniquely barcoded	Numerical analysis of transcripts	20000-200000	Macosko et al.,2015; Klein et al.,2015
Nanowell approaches	Custom nanowell chip: SeqWell Commercial platfroms: Nanogrid (ICell8) BD Rhapsody	1000s-10000s	The cells are partitioned into individual wells of a custom build nanowell chip and during reverse transcription cDNA molecules are unique	Numerical analysis of transcripts		Gierahn et al.,2017

V. SINGLE CELL PROTEOMICS IN PLANTS

While transcriptomic data provide valuable insights into gene activity, proteins are the true functional molecules within the cell, driving enzymatic reactions, signal transduction, structural organization, and stress responses. Because protein abundance does not always correlate with RNA levels, direct measurement of proteins at single-cell resolution is essential for understanding cellular behavior. However, single-cell proteomics presents substantial challenges in plants due to the extremely low quantities of protein available from individual cells and the limited sensitivity of many detection methods (Baker, 2012; Marx, 2019). Furthermore, the complex matrices of plant tissues—rich in secondary metabolites, lipids, and cell wall components—can interfere with protein extraction and quantification, emphasizing the need for highly optimized workflows.

Historically, protein analysis in single cells relied on antibody-based approaches, such as flow cytometry and immunofluorescence, which remain valuable tools for quantifying known proteins. Advanced flow cytometry platforms allow simultaneous detection of multiple protein

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markers using fluorophore-labeled antibodies, enabling high-throughput analysis of large cell populations (Perfetto et al., 2004). However, spectral overlap among fluorophores limits the number of measurable proteins, especially in complex tissues. The development of mass cytometry (CyTOF) addressed this constraint by using antibodies conjugated to rare heavy-metal isotopes, which are quantified by time-of-flight mass spectrometry. This method allows dozens of proteins to be measured in each cell without spectral interference and has expanded the potential of proteomic profiling in plant systems (Bodenmiller et al., 2012).

Another major advancement in the field is single-cell western blotting, which combines electrophoretic separation and immunodetection on microfabricated devices to measure protein abundance and isoforms from thousands of individual cells. This technique has proven useful for detecting post-translational modifications and for profiling both cytoplasmic and nuclear proteins (Hughes et al., 2014). Although most applications to date have been developed in animal systems, the method is increasingly being adapted for plant cells, especially protoplasts, where intact proteins can be extracted more readily.

For broader proteome coverage, researchers increasingly rely on mass spectrometry (MS)–based proteomics. Traditional MS requires microgram quantities of protein, far surpassing what a single cell can provide. However, recent innovations in sample preparation—such as the SP3 (single-pot solid-phase-enhanced sample preparation) method—enable protein capture and digestion from extremely small samples using paramagnetic beads (Hughes et al., 2014). When combined with high-sensitivity LC–MS/MS platforms, these protocols have made it possible to detect hundreds of proteins from minimal plant material, paving the way for true single-cell MS-based proteomics (Budnik et al., 2018). Although full proteome coverage at the single-cell level remains difficult in plants, rapid improvements in instrument sensitivity and peptide ionization are closing this gap.

Microfluidic technologies have further accelerated progress in single-cell proteomics. Integrated microchip systems can quantify dozens of intracellular and secreted proteins from thousands of cells in parallel, using minimal volumes of reagents and enabling real-time monitoring of cellular responses (Chattopadhyay et al., 2014). An important advantage of microfluidic platforms is their ability to maintain cell viability, allowing dynamic measurements of protein secretion following exposure to environmental or hormonal stimuli. For plant biology—where stress responses often depend on rapid shifts in protein abundance and post-translational regulation—such single-cell assays offer valuable opportunities to dissect complex signaling networks.

As the field matures, single-cell proteomics is expected to complement transcriptomic datasets, helping validate gene expression findings and refine cell-type classifications. Proteomic measurements will be particularly important for understanding pathways that are regulated primarily at the post-transcriptional level, including hormone perception, protein degradation, and rapid defense responses. In crop improvement research, single-cell proteomics holds promise for identifying biomarkers of stress tolerance and developmental traits that cannot be detected through genomic or transcriptomic analysis alone. Although technological barriers remain, the combination of microfluidics, advanced MS, and next-generation antibody technologies is rapidly transforming single-cell proteomics into an essential component of plant systems biology.

VI. SINGLE CELL METABOLOMICS IN PLANTS

The metabolome, which includes all low molecular weight metabolites produced by a cell, is a key indicator of cellular state, reflecting the precise metabolic activity and conditions within the cell. Due to the diversity and rapid dynamics of cellular metabolites, and the lack of tagging or amplification techniques for small molecules, measuring the metabolome at the single-cell level presents significant challenges (Zenobi, 2013).

Advancements in optical tools, such as genetically encoded optical nanosensors, along with improved expression systems and in vivo imaging techniques, have made real-time measurement of metabolites in single cells possible (Zenobi, 2013; Barros et al., 2013). These innovations enable the dynamic monitoring of metabolite levels in living cells, offering insights into cellular metabolism.

Single-cell mass spectrometry has also significantly advanced metabolomics research by enabling the analysis of metabolites within individual cells and subcellular structures. For instance, metabolites have been quantified in isolated neurons using single-cell capillary electrophoresis coupled with electrospray ionization time-of-flight mass spectrometry (Nemes et al., 2013). Furthermore, microarray platforms for mass spectrometry allow for the analytical validation of single-cell metabolites, which aids in monitoring cellular responses to environmental and genetic perturbations (Ibanez et al., 2013).

As technical advances continue, they are expected to improve the coverage of the metabolome, leading to more accurate and rapid identification of metabolites. These improvements will enhance our understanding of cell metabolism, allowing for the non-destructive measurement of metabolites in single cells, thereby refining the definition of cell state and function.

VII. SINGLE CELL EPIGENOMICS IN PLANTS

Epigenomic regulation plays a central role in shaping how plant cells interpret environmental cues and maintain stable patterns of gene expression across development. DNA methylation, histone modifications, chromatin accessibility, and nucleosome positioning collectively determine whether specific genomic regions are activated or silenced. Because these regulatory marks can vary substantially between

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individual cells—even within the same tissue—single-cell epigenomics provides an invaluable framework for understanding the molecular basis of cellular identity and plasticity (Ecker et al., 2012; Buenrostro et al., 2015). Bulk epigenomic assays obscure this variation by averaging signals across many cell types, thereby masking rare or transient regulatory states that are crucial for developmental and stress responses.

DNA methylation is one of the most studied epigenetic modifications in plants, with important roles in transposon silencing, genomic imprinting, and stress adaptation. Traditional whole-genome bisulfite sequencing provides high-resolution methylation maps, but single-cell applications require substantial adaptation due to the extremely low DNA content. Single-cell bisulfite sequencing (scBS-seq) and related methods have enabled researchers to examine methylation patterns at the level of individual nuclei, revealing heterogeneity in cytosine methylation across CG, CHG, and CHH contexts (Smallwood et al., 2014). These techniques have been applied to complex plant tissues to uncover cell-type–specific methylation signatures that correlate with transcriptional states and developmental processes (Lin et al., 2017). However, bisulfite-induced DNA degradation and amplification bias remain major limitations, prompting continued improvement of reaction chemistries.

Another important layer of epigenomic regulation involves chromatin accessibility, which determines whether transcription factors and polymerases can access DNA. Techniques such as Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) have been adapted for single cells, enabling genome-wide profiling of open chromatin regions from thousands of nuclei simultaneously (Buenrostro et al., 2015; Chen et al., 2018). Single-cell ATAC-seq has proven especially useful for dissecting regulatory networks in plant developmental systems, where transcription factor binding landscapes differ among cell types and developmental stages. For example, mapping chromatin accessibility in root tissues has revealed lineage-specific regulatory elements that direct differentiation and pattern formation (Jean-Baptiste et al., 2019).

Histone modifications add an additional layer of complexity to the regulatory landscape. Post-translational modifications such as H3K4me3, H3K27me3, and H3K9me2 influence chromatin compaction and transcriptional activation or repression. Although mapping histone marks traditionally requires large quantities of chromatin, miniaturized methods like CUT&Tag and CUT&RUN have recently been adapted to low-input samples, offering a path toward single-cell histone profiling in plants (O'Malley et al., 2016; Kaya-Okur et al., 2019). These techniques avoid the heavy cross-linking steps used in conventional chromatin immunoprecipitation, leading to higher signal-to-noise ratios and making them suitable for investigating chromatin states in rare or difficult-to-isolate plant cell types.

Epigenomic variation is particularly important in plant stress responses, where environmental challenges can induce rapid and reversible epigenetic changes. Single-cell epigenomic tools allow researchers to follow how individual cells acquire and maintain stress-related regulatory marks, helping explain why certain cell types exhibit heightened resilience or sensitivity (Ryu et al., 2019). Furthermore, the integration of single-cell epigenomic data with transcriptomic and proteomic datasets makes it possible to connect specific regulatory marks with functional outcomes, revealing how chromatin state transitions guide developmental or stress-induced reprogramming (Lin et al., 2017).

Although the field of plant single-cell epigenomics is still emerging, recent advances demonstrate its immense potential. Improvements in nuclei isolation, low-input library preparation, and combinatorial indexing are rapidly expanding the scale of epigenomic profiling. As these tools mature, they will enable researchers to construct comprehensive atlases of chromatin states across entire plant organs and developmental trajectories. Ultimately, single-cell epigenomics promises to deepen our understanding of plant regulatory biology and support efforts to develop crops that are more resilient, productive, and adaptable to environmental change.

VIII. APPLICATIONS OF SINGLE-CELL OMICS IN PLANTS

The integration of single-cell omics into plant biology has transformed the way researchers understand cellular specialization, developmental transitions, and stress adaptation. Because plant tissues contain highly diverse cell types that often undergo rapid physiological changes, resolving their individual molecular signatures has been essential for uncovering how complex biological processes unfold. Single-cell omics enables direct measurement of genomic, transcriptomic, proteomic, epigenomic, and metabolic variation at cellular resolution, offering insights that cannot be obtained from bulk tissue studies (Birnbaum, 2016; Efroni & Birnbaum, 2016).

One of the most significant applications of single-cell technologies has been the construction of cellular atlases for key developmental structures such as roots, shoots, and reproductive tissues. High-resolution transcriptomic maps have revealed extensive heterogeneity in the Arabidopsis root, identifying cell-type–specific regulators of differentiation and uncovering transcriptional gradients that shape tissue patterning (Denyer et al., 2019; Jean-Baptiste et al., 2019). These atlases have been instrumental in identifying rare cell populations, transitional states, and lineage trajectories that guide organ formation. Similar approaches applied to maize, rice, and tomato are now generating valuable resources for crop improvement (Shahan et al., 2022; Xu et al., 2021).

Single-cell omics has also deepened understanding of plant regeneration and developmental plasticity, areas where plants exhibit remarkable flexibility. Studies of root regeneration demonstrate that individual cells can rapidly re-establish stem cell niches and reprogram transcriptional networks following injury, mirroring early embryonic patterning (Efroni et al., 2016). These findings highlight the

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dynamic regulatory capacity of plant cells and illustrate how single-cell analyses can clarify the molecular cues that direct tissue repair and organogenesis.

In the context of stress biology, single-cell transcriptomics and epigenomics have resolved long-standing ambiguities regarding hormone signaling pathways. Bulk studies of ethylene, jasmonate, salicylic acid, and abscisic acid often produced conflicting results because responses differ among cell types. Single-cell profiling has revealed that hormone interactions are highly compartmentalized, with specific cell populations exhibiting distinct activation patterns under drought, salinity, or pathogen attack (Coolen et al., 2016; Ryu et al., 2019; Shaw et al., 2021). These insights are guiding more accurate models of stress signaling and informing strategies for engineering stress-resilient crops.

A rapidly emerging application is the use of single-cell genomics in reproductive biology, particularly through sequencing individual pollen nuclei. This approach allows direct assessment of recombination events, haplotypes, and allele frequencies without the need to generate large segregating populations. As demonstrated in barley, maize, and Arabidopsis, pollen sequencing accelerates quantitative trait locus (QTL) mapping and enables fine-scale analysis of meiotic recombination landscapes (Dreissig et al., 2017; Morris et al., 2020). Such insights are valuable for breeding programs targeting yield, disease resistance, and environmental tolerance.

Single-cell proteomics and metabolomics contribute additional layers of functional information. Cellular-level protein profiling helps elucidate post-transcriptional regulation, signaling dynamics, and rapid defense responses that cannot be captured by transcriptomics alone (Hughes et al., 2014; Bodenmiller et al., 2012). Likewise, single-cell metabolomics reveals biochemical heterogeneity within tissues, uncovering metabolic signatures that define specialized cells such as guard cells, root hairs, and trichomes (Zenobi, 2013; Lapainis et al., 2009). These insights deepen understanding of metabolic specialization and resource allocation strategies.

Finally, multi-omics integration represents one of the most powerful applications of single-cell technologies. By combining transcriptomic, proteomic, epigenomic, and metabolomic data, researchers can reconstruct regulatory networks that govern cell identity and environmental responses with unparalleled resolution (Lin et al., 2017). These integrative approaches are critical for linking gene expression to biochemical outcomes and for identifying master regulators of developmental and stress-associated pathways.

Overall, the application of single-cell omics in plants is reshaping fundamental and applied research. As technologies continue to improve in throughput, cost, and sensitivity, single-cell analyses are expected to play an increasingly central role in crop improvement, systems biology, and the study of plant adaptation to global climate challenges.

IX. APPLICATION OF SINGLE-CELL TECHNOLOGY IN PLANTS

Single-cell technology holds immense potential in plant research, although examples of its application are still relatively scarce. Genotyping, developmental studies, and cell typing using single-cell approaches in animal and human samples are highly relevant and extensively used for analyzing biomolecule synthesis and interactions (Efroni et al., 2017). However, in plants, the presence of a rigid cell wall and the lack of suitable protocols for rapid tissue dissociation present significant challenges. While a few cell types in multicellular plants can be dissociated without enzymatic treatment, most require enzymatic or mechanical cell wall digestion, which often stresses the cell protoplasts, leading to negative impacts on gene expression. Despite these challenges, studies on cell identity in *Arabidopsis thaliana* have been successfully conducted using single-cell transcriptome amplification methods compatible with protoplast amplification (Efroni et al., 2015).

One of the most promising applications of single-cell genome sequencing is in pollen typing, particularly in molecular genetics and agricultural breeding. During meiosis, chromatids recombine, leading to genetic differences in daughter cells. The frequency of allele segregation in pollen grains influences genetic diversity and the distribution of beneficial traits (e.g., crop yield) to offspring plants. Traditional studies of plant population genomics require analyzing large numbers of offspring plants using low-throughput cytological assessments and conventional breeding, which is slow and costly due to the long generational time of many plants (e.g., wheat can take up to 9 months to mature in the field).

Single-cell genome sequencing of pollen grains offers an efficient alternative, as it can haplotype the parental chromosomal contribution and help understand the factors regulating crossing-over frequency. Pollen typing requires only one plant and is more efficient than fluorescence-activated cell sorting (FACS). It is particularly useful in quantitative trait loci (QTL) mapping, which typically requires thousands of replicates (Chen et al., 2008). For example, studies on barley pollen have shown that the majority of recombination sites are located at the distal ends of chromosomes, despite the presence of "peri-centromeric" regions (Dreissig et al., 2017). By linking whole-genome sequencing with other "omics" data, such as transcriptomics and proteomics, single-cell multi-omics approaches can further enhance our understanding of plant meiosis and pollen formation.

X. SINGLE CELL PLATFORMS IN PLANTS

The rapid expansion of single-cell omics in plant research has been driven by major advancements in experimental platforms, sequencing chemistries, and computational tools. Since plant cells present unique technical barriers—including rigid cell walls, heterogeneity in cell size, abundant secondary metabolites, and complex genomic architectures—specialized platforms have been

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essential for adapting these methods to plant systems (Birnbaum, 2016; Gawad et al., 2016). Over the past decade, innovations in microfluidics, low-input sequencing, and nucleus-based approaches have made high-resolution cellular profiling increasingly feasible across a wide range of plant tissues.

A central component of modern single-cell technology is droplet-based microfluidics, which enables massive parallelization of transcriptome and chromatin profiling. Platforms such as Drop-seq, inDrop, and the 10x Genomics Chromium system use microfluidic channels to encapsulate individual cells or nuclei into nanoliter droplets along with barcoded beads. These systems can profile tens of thousands of cells in a single experiment, making them ideal for constructing cellular atlases of roots, leaves, and developing organs (Klein et al., 2015; Macosko et al., 2015; Shulse et al., 2019). Although most droplet platforms were developed for mammalian cells, they have been successfully adapted for plant protoplasts and nuclei, provided that efficient cell- or nucleus-isolation protocols are available (Denyer et al., 2019; Farmer et al., 2021).

In tissues where generating viable protoplasts is difficult—such as lignified stems, mature leaves, and reproductive structures—single-nucleus sequencing platforms offer a powerful alternative. Single-nucleus RNA sequencing (snRNA-seq) and single-nucleus ATAC-seq (snATAC-seq) bypass the need for enzymatic digestion by using mechanical disruption to release nuclei. This approach reduces stress-induced transcriptional artifacts and enables profiling of cell types that are otherwise inaccessible (Sunaga-Franze et al., 2022; Schon et al., 2021). The compatibility of nuclei isolation with flash-frozen or fixed tissues also expands the types of plant samples that can be analyzed, including field-grown crops.

Alongside these high-throughput systems, nanowell-based platforms such as Seq-Well, BD Rhapsody, and ICell8 allow researchers to capture single cells in microwells before barcoding their RNA or chromatin. Although these platforms often process fewer cells than droplet systems, they provide greater experimental control, including imaging-based quality assessment of captured cells (Gierahn et al., 2017). Their flexibility makes them well suited for specialized tissues or rare cell populations where sample quantity is limited.

The rise of combinatorial indexing approaches has also expanded single-cell analysis in plants. Methods such as sci-RNA-seq, sci-ATAC-seq, and SNARE-seq avoid physical isolation of individual cells by using multiple rounds of barcoding to uniquely label nucleic acids from thousands of nuclei (Cao et al., 2017; Cusanovich et al., 2015). These high-throughput strategies reduce cost and eliminate the need for microfluidic devices, making them attractive for large-scale profiling of complex plant tissues.

Improvements in sequencing chemistries, including template-switching reverse transcriptases, low-input amplification kits, and high-sensitivity library preparation methods, have further increased the resolution of plant single-cell omics. Tailored reagents for GC-rich or repetitive genomes, along with polymerases that minimize amplification bias, are becoming increasingly important as single-cell methods expand to polyploid crops such as wheat, barley, and sugarcane (Wendel et al., 2016).

In parallel with wet-lab innovations, computational platforms have become indispensable for processing and interpreting single-cell data. Tools such as Seurat, Scanpy, and Monocle enable clustering, trajectory inference, differential expression analysis, and integration of multi-omics datasets (Butler et al., 2018; Wolf et al., 2018; Trapnell et al., 2014). For plant-specific challenges, customized pipelines have been developed to address issues such as polyploidy, cell-type annotation, and organ-specific transcriptional gradients. These tools provide the framework for building reference atlases and identifying regulatory networks underlying development and stress responses.

Collectively, these technological platforms have established a strong foundation for single-cell omics in plant research. Continued improvements in microfluidics, nuclei-based sequencing, combinatorial indexing, and computational integration are expected to make single-cell profiling increasingly accessible, even for complex crop species. As these technologies mature, they will enable multidimensional characterization of plant growth, adaptation, and cellular diversity at an unprecedented level of detail.

XI. DATA REPOSITORIES AND RESOURCES FOR PLANT SINGLE-CELL OMICS

As single-cell omics technologies continue to mature, the volume of data generated from plant systems has expanded dramatically. Organizing, curating, and sharing these datasets through publicly accessible repositories is essential for enabling cross-study comparisons, meta-analyses, and the development of integrative multi-omics frameworks. Data repositories not only preserve raw sequencing data but also provide annotated gene-expression matrices, metadata, and analytical tools that support reproducibility and accelerate discovery (Regev et al., 2017; Lin et al., 2017).

Most raw single-cell sequencing data are deposited in international archives such as the NCBI Gene Expression Omnibus (GEO) and the European Nucleotide Archive (ENA), which serve as major hubs for transcriptomic and epigenomic datasets from both model and crop plants. These repositories allow researchers to access processed count matrices, metadata on cell isolation and library preparation methods, and detailed information about experimental conditions. For example, GEO has hosted datasets from landmark studies profiling the Arabidopsis root, maize leaf, and rice seedling using both protoplast-based and nucleus-based approaches (Denyer et al., 2019; Shulse et al., 2019; Xu et al., 2021). The availability of these datasets has facilitated the development of computational benchmarks and comparative analyses across species.

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In addition to general repositories, specialized platforms have emerged to support plant-specific single-cell research. The Single Cell Expression Atlas, hosted by EMBL-EBI, provides standardized processing pipelines and interactive visualization tools that allow researchers to explore gene-expression patterns across various plant tissues and developmental stages. Its curated datasets offer valuable resources for identifying marker genes, comparing transcriptional programs, and analyzing cellular differentiation pathways. Similarly, community-driven resources such as the Plant Cell Atlas initiative aim to integrate transcriptomic, proteomic, imaging, and spatial data to build comprehensive maps of plant cellular organization (Birnbaum, 2016).

As epigenomic and multi-omics studies grow in popularity, corresponding repositories are expanding as well. Platforms such as Cistrome, SRA, and PlantRegMap store chromatin accessibility datasets, transcription factor–binding profiles, and regulatory element annotations derived from ATAC-seq, ChIP-seq, CUT&Tag, and combinatorial indexing experiments (O'Malley et al., 2016; Kaya-Okur et al., 2019). These resources provide essential context for linking gene-expression changes to underlying regulatory mechanisms and offer reference maps for interpreting cell-type–specific epigenomic variation.

Computational tools integrated within these repositories further enhance their utility. Frameworks such as Seurat, Scanpy, and Monocle have been adapted for plant datasets, allowing researchers to perform clustering, trajectory inference, and cell-type annotation directly from public data (Butler et al., 2018; Wolf et al., 2018; Trapnell et al., 2014). Plant-specific annotation tools and curated marker gene lists have also become more common, reflecting community efforts to standardize analytical pipelines.

These repositories and tools form the foundation for open, collaborative research in plant single-cell biology. By providing access to standardized datasets and computational resources, they enable researchers to validate findings, compare across species, and build integrated models of plant development and stress adaptation. As the diversity and scale of plant single-cell data continue to expand, these platforms will play an increasingly important role in advancing systems-level understanding and supporting crop improvement efforts.

XII. CONCLUSION

The emergence of single-cell omics has fundamentally reshaped plant biology by providing a powerful framework for exploring cellular diversity, developmental trajectories, and stress-responsive behaviors at unprecedented resolution. Unlike bulk analyses, which average signals across complex tissues, single-cell approaches allow researchers to capture the molecular identities of individual cells and uncover the heterogeneity that underlies organ formation, physiological plasticity, and environmental adaptation (Efroni & Birnbaum, 2016; Regev et al., 2017). By integrating information from genomes, transcriptomes, proteomes, epigenomes, and metabolomes, single-cell technologies offer a multidimensional understanding of how plant cells perceive signals, regulate gene expression, and execute specialized functions.

Advances in protoplast isolation, nuclear extraction, droplet microfluidics, combinatorial indexing, and low-input sequencing methods have greatly expanded the range of plant tissues that can be profiled at single-cell resolution (Denyer et al., 2019; Farmer et al., 2021). As a result, high-resolution cellular atlases have been constructed for roots, leaves, meristems, and reproductive structures, revealing novel cell types, lineage relationships, and gene-regulatory networks (Jean-Baptiste et al., 2019; Shulse et al., 2019). These resources are beginning to inform genetic engineering and breeding strategies by identifying molecular regulators that underlie desirable agronomic traits such as stress tolerance, nutrient efficiency, and developmental robustness.

The integration of single-cell epigenomics, proteomics, and metabolomics with transcriptomic data further strengthens the potential of these technologies. Emerging multi-omics approaches link chromatin states with gene expression patterns, protein activity, and metabolic outputs, providing comprehensive insight into the regulatory logic of plant cells (Lin et al., 2017; Kaya-Okur et al., 2019). Such integrative frameworks are poised to deepen our understanding of how plants reorganize molecular networks in response to biotic and abiotic stress, contributing to efforts to enhance climate resilience in crops.

Although challenges remain—including optimization of cell isolation protocols, improved sensitivity for proteomic and metabolomic detection, and the need for plant-specific computational tools—the field is advancing rapidly. Data repositories such as GEO, ENA, and the Single Cell Expression Atlas are expanding access to high-quality datasets, supporting cross-species comparisons and accelerating discovery (O'Malley et al., 2016; Birnbaum, 2016). Continued innovations in instrumentation, sequencing chemistry, and bioinformatic analysis will further democratize single-cell approaches, allowing researchers to apply these methods to more diverse plant species and environmental conditions.

In summary, single-cell omics represents a transformative advance in plant science. By illuminating the molecular complexity of individual cells and capturing the dynamic processes that drive development and adaptation, these technologies lay the foundation for next-generation crop improvement and fundamental discoveries in plant biology. As methodological barriers continue to diminish and multi-omics integration becomes more routine, single-cell approaches will remain at the forefront of efforts to understand—and ultimately engineer—the cellular basis of plant form and function.

➢ *Authors Contribution Statement*

- ST: The author investigated, wrote and conceptualized the article. SG supervised, reviewed and edited the article.

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