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Title

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Journal

Trends in Plant Science, 29(12)

ISSN

1360-1385

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Publication Date

2024-12-01

DOI

10.1016/j.tplants.2024.06.008

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Trends in Plant Science

Review

Unraveling plant–microbe symbioses using single-cell and spatial transcriptomics

Karen Serrano [1,2,3,5], Francesca Tedeschi [4,5], Stig U. Andersen [4,*], and Henrik V. Scheller [1,2,3,*]

Plant–microbe symbioses require intense interaction and genetic coordination to successfully establish in specific cell types of the host and symbiont. Traditional RNA-seq methodologies lack the cellular resolution to fully capture these complexities, but single-cell and spatial transcriptomics (ST) are now allowing scientists to probe symbiotic interactions at an unprecedented level of detail. Here, we discuss the advantages that novel spatial and single-cell transcriptomic technologies provide in studying plant–microbe endosymbioses and highlight key recent studies. Finally, we consider the remaining limitations of applying these approaches to symbiosis research, which are mainly related to the simultaneous capture of both plant and microbial transcripts within the same cells.

Highlights

Beneficial plant–microbe interactions are critical to plant productivity in both natural and agricultural ecosystems due to benefits such as improved plant nutrition and abiotic stress tolerance.

The legume–rhizobia symbiosis and plant–arbuscular mycorrhizal fungi symbiosis both involve continuous signaling and coordination between two organisms.

Within symbioses, the development of novel microbial and plant structures is driven by symbiosis-specific gene expression in a few specific cell populations.

Genetic engineering to take better advantage of these interactions relies on the identification of symbiosis-responsive plant and microbial genes that can serve as targets for modification.

Applying single-cell and spatial RNA-seq to study plant–microbe endosymbiosis can facilitate the identification of such genes and improve our general understanding of the complex biology of symbiotic interactions.

Transcriptomic investigations of plant–microbe symbioses

The field of plant **transcriptomics** has experienced rapid growth and comprehensive reviews describe recent developments in plant single-cell transcriptomes [1–5]. Technological advances in single-cell RNA-seq (scRNA-seq) and spatial RNA-seq present new opportunities for research into complex plant–microbe interactions. scRNA-seq allows the study of gene expression occurring in individual cells rather than whole tissue, providing more detail on heterogeneous cell populations. scRNA-seq can be applied to investigate the response of individual plant cells to symbiotic infection, revealing cell-type-specific gene expression. ST enables analysis of individual cells in fixed positions within the tissue, providing the physical location of gene expression [6]. This methodology provides insight into the spatial organization of plant and symbiont gene expression during symbiosis. In this review, we focus on the utilization of high-throughput scRNA-seq and spatial RNA-seq technologies to investigate beneficial plant–microbe interactions.

Open questions in plant–microbe endosymbiosis

Plant species engage in mutually beneficial interactions with a wide array of microorganisms. A prevalent characteristic of such symbioses is the microorganisms' capacity to promote plant growth by facilitating the acquisition of scarce nutrients. By trading these nutrients, the microbe receives carbon generated by the plant. The legume–rhizobial bacteria and the plant–**arbuscular mycorrhizal fungi (AMF)** (see Glossary) interactions are two of the most well-explored endosymbioses, characterized by intracellular accommodation of the symbiont (Figure 1, Key figure).

The legume–**rhizobia** interaction is characterized by a complex biological process leading to the formation of specialized structures called **nodules** where the bacteria reside and convert atmospheric nitrogen into a form usable by plants. Nodulation has been extensively investigated at cellular, molecular, and physiological levels and requires the activation of temporally and spatially coordinated programs in a limited number of root cells [7,8]. This involves sophisticated communication, mediated by **flavonoids** and nodulation (Nod) factors. Legumes release flavonoids into the soil to recruit potential symbiotic partners. Rhizobia sense flavonoids and produce **Nod factors** in response. Nod factors, in turn, trigger a response in the root that leads to root hair curling and

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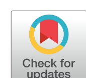

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Key figure

Transcriptomics as a tool for plant–microbe endosymbioses

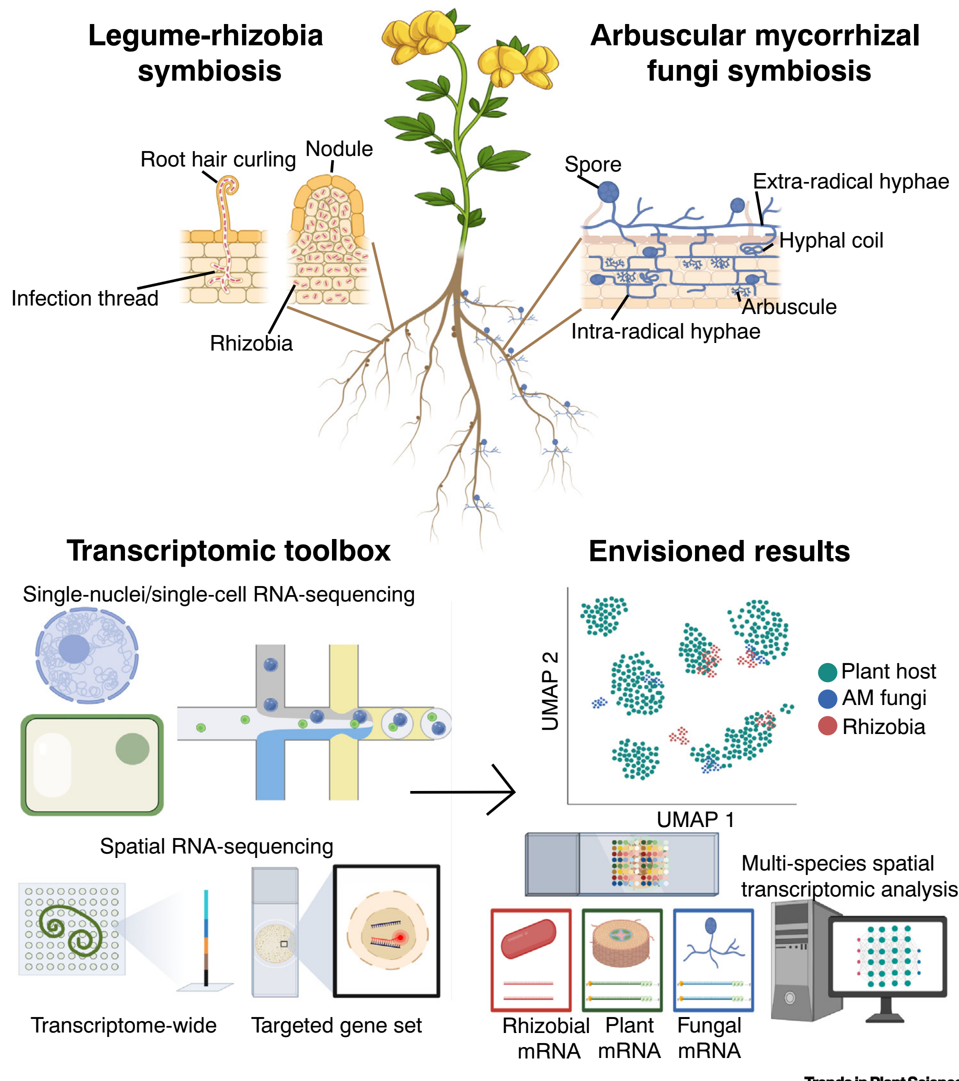

Figure 1. An overview of the legume–rhizobia and plant–arbuscular mycorrhizae (AM) symbioses and the corresponding microbial structures. The legume–rhizobia symbiosis is characterized by rhizobial infection of root cells that causes curling of root hairs and the development of infection threads. Infection threads extend through the root hair into cortical cells and culminate in the formation of specialized structures called nodules in which bacteria convert atmospheric nitrogen into a form usable by plants. The legume rewards the bacteria with carbon. The AM fungi–plant symbiosis involves controlled intracellular and intercellular fungal hyphal colonization of the plant root and culminates in the formation of arbuscules, highly branched fungal hyphal structures in which soil nutrients are transferred from the fungus to the plant host in exchange for carbon. Single-cell and single-nucleus RNA-seq technologies and both targeted and non-targeted spatial RNA-seq technologies have evolved over recent years and hold great potential as tools to study the complexities of plant–microbe endosymbioses. The ultimate goal of such studies is to simultaneously capture microbial endosymbiont and plant host RNA during these interactions to study cell-type-specific infection expression patterns in a spatially resolved manner. Figure created with BioRender.com.

Glossary

Arbuscular mycorrhizal fungi (AMF): a type of soil fungus that can colonize the roots of most vascular plants to form arbuscules and transfer soil nutrients to the plant host in exchange for carbon.

Arbuscules: highly branched fungal hyphal structures that form within root cortical cells and are the site of nutrient transfer between mycorrhizal fungi and host plants.

Barcode: a segment of short sequence of DNA that is used to identify or localize a specific molecule of interest.

Flavonoids: a group of polyphenolic secondary metabolites that initiate and regulate nodulation. Legume roots release flavonoids into the rhizosphere to signal rhizobia and promote their production of nodulation factors.

Infection thread (IT): a plant-host-derived tubular compartment that allows apoplastic infection of the plant by rhizobia.

***In situ* hybridization (ISH):** a within-tissue nucleic acid detection and localization technique that relies on labeled probes with sequences complementary to the target nucleic acid.

Myc factors: refers to both chitooligosaccharides and lipochitooligosaccharides (LCOs) secreted by AMF to trigger plant symbiotic responses.

Nod factors: diffusible LCO compounds released by rhizobia in response to flavonoids present in root exudate and recognized by the legume plant to activate a symbiosis signaling pathway.

Nodules: specialized structures that form on the roots of legumes through a symbiotic association with soil bacteria known as rhizobia. Rhizobia fix atmospheric nitrogen gas into ammonia in nodules, subsequently utilized in the synthesis of amino acids and nucleotides.

Protoplast: an isolated cell created by mechanical or enzymatic removal of the surrounding cell wall.

Pseudotime analysis: computationally placing cell data from a scRNA-seq library along a simulated temporal trajectory based on how their transcriptional profiles progress to model the real-time progression of a target biological process.

Resolution: refers to the size of capture spots or voxels in spatial transcriptomic technologies, which translates to the scale of cellular detail that the methodology is able to profile.

culminates in the formation of an infection pocket and the development of **infection threads (ITs)**. ITs extend through root hairs towards cortical cells and ramify in nodule primordia, which are formed by dividing cortical cells, facilitating the release of rhizobia into nodules. The perception of rhizobia in root hairs of legumes such as *Phaseolus*, *Glycine*, and *Lotus* spp. induce the formation of nodules that lose their meristematic activity over time (determinate nodules), while other legumes like *Pisum sativum* and *Medicago truncatula* form indeterminate nodules with a persistent meristem [9].

What are the requirements for successful intracellular rhizobial infection? How does the plant decide which root hair among thousands should respond to the rhizobia facilitating the initiation or progression of intracellular ITs? Classical transcriptomic approaches applied to whole roots and root hairs of legume models have brought us closer to answering these questions [10,11]. These studies were able to capture infected cells but could not distinguish between the transcriptional signatures of root hairs that were successful in IT formation and those that were unsuccessful but still exhibited a symbiotic response.

The plant–AMF symbiosis is defined by the controlled fungal colonization of roots, which culminates in both extra- and intraradical hyphal networks as well as branched structures called **arbuscules** in cortical cells that facilitate metabolite transfer [12]. Intercellular passage and subsequent intracellular accommodation of the fungus involves novel gene expression in both colonized and non-colonized adjacent plant cells and continuous signaling between the partners [13–15]. Nutrient-stressed plant roots exude **strigolactones** into the soil, which stimulate AMF spore germination and hyphal branching towards the root [16,17]. AMF exude **Myc factors** [18] in response, which trigger plant transcriptional and physiological responses. Following physical contact, hyphae enter the epidermal cell layer and subsequently travel inter- and intracellularly to the inner cortical cells in which arbuscules will form [19]. Once inside the cortical cell, the fungus penetrates the plant cell wall and the plant synthesizes a new peri-arbuscular membrane to surround the arbuscule, equipped with proteins responsible for facilitating metabolite exchange [19–21]. A suite of physiological changes occurs in the cell to accommodate and maintain the arbuscule, including, but not limited to, a reduction in vacuole size and organelle compaction [21]. Arbuscules are transient structures [22]; thus, the window for this exchange is limited.

Due to asynchronous colonization, multiple fungal structures exist within different cell types simultaneously, which precludes efforts to tackle the complexities of the transcriptional programs involved in this symbiosis with traditional RNA-seq methods. How can the individual stages of arbuscular mycorrhizal symbiotic development be distinguished when they occur simultaneously in the root tissue? How do colonized cells and adjacent non-colonized cells differ in their transcriptional signatures? How does the fungus control its development in the root and subsequent metabolite transfer from the soil? Many traditional transcriptomic studies of AMF-inoculated and mock-inoculated roots from many different plant and fungal partners have identified thousands of differentially regulated genes under mycorrhizal conditions [23–32]. Insights from studies utilizing laser-capture microdissection [14,15] have revealed the importance of including non-colonized cells adjacent to arbusculated cells. Furthermore, research regarding the genetic landscape and expression patterns of AMF [33–42] has greatly expanded our knowledge of the symbiont in this interaction. Advances in RNA-seq technology will enable research into cell-type-specific responses from both partners.

The commonalities between these endosymbioses continue to emerge, supporting the theory that rhizobia co-opted existing cellular programs for the AMF symbiosis for bacterial accommodation [43,44]. Due to large differences in culturability between bacteria and fungi, research into

Rhizobia: a type of soil bacteria that can colonize the roots of legume plants to form root nodules and fix atmospheric nitrogen for the plant host in exchange for carbon.

RNA-seq library: a pool of DNA fragments from an RNA-seq experiment that contain barcodes and adapter sequences specific to each sequencing platform.

Strigolactones: carotenoid-derived phytohormones that function as signaling molecules and promote the branching of mycorrhizal hyphae.

Transcriptomics: the study of an organism's transcriptome, which includes all genomic transcripts in various forms of RNA. This is also commonly referred to as 'RNA-seq'.

Unique molecular identifiers (UMIs): index tags added to each molecule in sequencing libraries to distinguish between them and to more accurately quantify the number of molecules in a sample.

Voxel: the 3D analog of a pixel; refers to the individual capture oligo spot on a slide-based spatial transcriptomic technologies.

endosymbioses has focused on the legume–rhizobia symbiosis. The common symbiotic signaling pathway (CSSP), in particular, has been the focus as it includes genes with similar functions in both symbioses [7,45]. However, additional genes with roles in both symbioses that fall outside this pathway and numerous genes within the pathway with fine distinctions in their functions exist in either pathway [45]. The details of how these two systems overlap and how each influences the other during co-infection are likely to be revealed with further transcriptomic analysis.

A transcriptomic toolbox for symbiosis research

Novel transcriptomics technologies are revolutionizing gene expression analysis, providing unprecedented insights into the complexity of biological systems. These advances mark a step forward to uncover fundamental cellular and molecular processes in symbiosis. However, as RNA-seq methodologies continue to rapidly diversify across various platforms, it is important to understand the unique features and applications of each method. This knowledge is crucial to make an informed choice on how to most effectively answer open questions in plant–microbe interactions.

scRNA-sequencing involves key steps such as single-cell (or single-nucleus) isolation and capture, cell lysis, reverse transcription, cDNA amplification, and **RNA-seq library** preparation. The first step, separating and isolating intact plant **protoplasts** or nuclei, is critical. If the integrity of these biological entities is compromised, this could decrease the detection of **unique molecular identifiers (UMIs)** and genes. There are distinct differences between cellular and nuclear transcriptomes in plants. The cellular transcriptome contains transcripts from the nuclear and organellar compartments, while the nuclear transcriptome is less complex and contains polyadenylated mRNA transcripts and rRNAs. Thus, while nucleus extraction is often quicker and more adaptable, the nuclear transcriptome may fail to capture important biological processes involved in mRNA processing, RNA stability, and metabolism [2,46]. Protoplasting of plant tissues, however, involves cell-wall-degrading enzymes, which may induce artificial stress responses. Additionally, Van den Brink and colleagues [47] revealed dissociation-induced transcriptome changes as the potential outcome of the physical dissociation process during single-cell isolation in animal cells. To test the validity of protoplasting, researchers combined the scRNA-seq data for all captured cells from plant root into a pseudo-bulk dataset and compared this dataset with a conventional bulk RNA-seq dataset of non-protoplasted plant root tissues and found that the two datasets were highly correlated with each other, regardless of the presence or absence of protoplasting-induced genes [48,49]. Despite their potential limitations, both scRNA-seq and single-nucleus RNA-seq (snRNA-seq) are valid methodologies.

To date, the main high-throughput technology for sn/scRNA-seq in plant single-cell transcriptomics is Chromium, a microfluidics-based method provided by 10X Genomics [50]. In brief, single cells or single nuclei are encapsulated along with gel-bead-containing barcoded oligonucleotides, reagents, and oil to create gel beads in emulsion (GEMs) in which the reverse transcription of polyadenylated mRNA occurs. When the GEMs are broken and the barcoded cDNAs are released, the entire cDNA content of a single cell or nucleus will have the same **barcode**, allowing the sequencing reads to be mapped back to their original single cell/nucleus of origin (https://www.10xgenomics.com). Emerging technologies that do not rely on complex instruments have been developed with the prospect of reducing costs and increasing accessibility. Particle-templated instant partitions (PIPseq™) can simultaneously segregate complex cell mixtures into partitions with barcoded template particles that can be processed for scRNA-seq (https://www.fluentbio.com/technology/). Another rising technique is Evercode™ from Parse Bioscience. The Evercode™ combinatorial barcoding technology uses particle-templated emulsification to enable single-cell encapsulation and barcoding of cDNA in droplet emulsions, providing a simple, flexible, and scalable next-generation workflow for scRNA-seq (https://www.parsebiosciences.com/technology).

ST enables the preservation of a cell's position and thus spatial tracking of gene expression [51]. Plant–microbe endosymbioses are restricted to specific cell types and manifest in various unique symbiotic structures within and between plant cells. Therefore, it is critical to preserve the spatial landscape. There are many reviews that cover all recently developed spatial technologies [52–55], but here we focus on two main types: (i) spatial barcoding-based transcriptome wide; and (ii) targeted ***in situ* hybridization (ISH)** based.

Untargeted technologies include ST [56], Slide-Seq/V2 [57,58], high-definition ST (HDST) [59], deterministic barcoding in tissue (DBIT-seq) [60], Seq-Scope [61], and Stereo-Seq [62] (Table 1). All of these allow transcriptome-wide capture of mRNA transcripts from fresh-frozen or formalin-fixed paraffin-embedded tissue sections and rely on positional next-generation sequencing to generate spatially resolved transcriptomic libraries. These technologies differ greatly in their **resolution**, capture efficiency, and accessibility, all of which can significantly impact the quality of the resulting libraries [63]. ST, first developed in 2016 and commercialized in 2018 by 10X Genomics as Visium, is the most widely used. Tissue sections are fixed to spatial gene expression slides engineered to enable spatially barcoded mRNA capture from ~5000 **voxels**. Cells are permeabilized directly on the slide, releasing mRNA onto the capture oligos within the voxels, and reverse transcription and subsequent cDNA library construction is performed. Given that Visium is commercially available and relatively adaptable, the main limitation

Table 1. Overview of transcriptome-wide spatial methodologies

Technology	Methodology	Commercially available?	Capture area size	Resolution/detection limit (ISH)	Refs
ST	Untargeted, transcriptome-wide capture	Visium by 10X Genomics	6.5 × 6.5 mm	55 μm	[56]
Slide-Seq	Untargeted, transcriptome-wide capture	See Slide-Seq V2	66 tissue sections over 39 × 39 mm^2	10 μm	[57]
HDST	Untargeted, transcriptome-wide capture	N/A	5.7 × 2.4 mm	2 μm	[59]
DBIT-seq	Untargeted, transcriptome-wide capture	AtlasXomics	3.8 × 3.8 mm	50, 25, and 10 μm	[60]
Seq-Scope	Untargeted, transcriptome-wide capture	N/A	0.8 × 1 mm	0.6 μm	[61]
Slide-Seq V2	Untargeted, transcriptome-wide capture	Seeker by Curio Bioscience	3 × 3 mm or 10 × 10 mm	10 μm	[58]
Stereo-Seq	Untargeted, transcriptome-wide capture	STOmics by BGI	13.2 × 13.2 cm	0.22 μm	[62]
MERFISH	Targeted, ISH	MERSCOPE by Vizgen	2 × 1.5 cm	10 000 genes	[64]
seqFISH	Targeted, ISH	See seqFISH+	0.5 × 0.5 mm	250 genes	[65]
seqFISH+	Targeted, ISH	GenePS by Spatial Genomics	1 × 1 mm	10 000 genes	[66]
Molecular Cartography	Targeted, ISH	Molecular Cartography by Resolve Biosciences	26 × 26 mm	300 nm/100 genes	[67]
DSP	Targeted, ISH	GeoMX DSP by NanoString	35.3 × 14.1 mm	50 μm/whole transcriptome	[68]
Split-FISH	Targeted, ISH	N/A	Varies, 3 × 3 mm in original study	317 genes	[69]
EEL-FISH	Targeted, ISH	Esper High Plex Assay by Rebus Biosystems	24 × 24 mm	5000 genes	[70]
PHYTOMap	Targeted, ISH	N/A	Whole mount	100 genes	[71]

of this method is the resolution of ~55 μm, which for many tissues is above single cell. To date, two untargeted technologies can achieve submicrometer resolution: Seq-Scope and Stereo-Seq. Seq-Scope indirectly measures mRNA at ~0.6-μm resolution [61]. Another advantage of Seq-Scope is that its high capture efficiency (~4700 UMIs/cell) is comparable with that of scRNA-seq methodologies [61]. Stereo-Seq, or spatial enhanced-resolution omics sequencing, uses DNA nanoball (DNB) technology for RNA capture [62]. Stereo-Seq achieves the highest density of capture spots and highest resolution of all spatially barcoded methods [62].

ISH-based methodologies all rely on direct labeling of transcripts in tissue sections to detect target gene expression. The main methodologies include MERFISH (MERSCOPE) [64], seqFISH/seqFISH+ [65,66], Molecular Cartography [67], digital spatial profiling (DSP) [68], Split-FISH [69], EEL-FISH [70], and plant hybridization-based targeted observation of gene expression map (PHYTOMap) [71] (Table 1). Building off the original single-molecule FISH (smFISH) technology [49,72], two methodologies were developed to allow simultaneous detection of dramatically more RNA molecules: seqFISH/seqFISH+ and MERFISH (now commercially available as MERSCOPE). Molecular Cartography is another commercially available smFISH-based technology. It has high sensitivity and is currently limited to a panel of 100 genes [67]. Last, PHYTOMap was developed specifically for whole-mount plant tissue. In PHYTOMap, gene-targeted DNA probes are hybridized to targets directly within fixed whole-mount plant tissues and amplified *in situ* [71].

Decoding symbiosis: transcriptomic research on the legume–rhizobial and plant–mycorrhizal interactions

Pioneer scRNA-seq work in *Arabidopsis thaliana* roots [49,73–77] paved the way for the transcriptomic investigation of various biological processes across diverse plant species. More recently, scRNA-seq and spatial RNA-seq transcriptomics have been applied to study plant–microbe endosymbioses (Figure 2 and Table 2). Cervantes-Pérez *et al.* applied snRNA-seq to *M. truncatula* roots inoculated with the rhizobium *Ensifer meliloti* at 2 days post-inoculation (dpi) [78]. This provided a comprehensive annotation of *M. truncatula* root cell type as well as an analysis of the transcriptomic response of cells to rhizobial infection. Gene expression patterns were unique to specific cell types, indicating a cell-type-specific role for certain genes in nodulation. This study confirmed decades of research in legume nodulation, identifying key genes and pathways involved in nodulation, including genes related to cell division, signaling, and nutrient transport [78]. Moreover, it led to the discovery of genes that had not been previously highlighted by bulk transcriptome analyses. Another scRNA-seq analysis in *M. truncatula* infected with *E. meliloti* was conducted by Ye and colleagues using indeterminate *M. truncatula* nodule protoplasts at 14 dpi [79]. This study improved our understanding of the early stages of root nodulation through the identification and characterization of 13 distinct nodule cell clusters. Additionally, **pseudotime analysis** revealed that two groups of apical meristematic cells diverge into symbiotic and nonsymbiotic fates. Last, investigation of nitrogen assimilation in nodules provided insight into how uninfected cells may play a role in the overall nodule functioning. Taking a new approach, Liu and colleagues focused on the earliest stages of signaling between plants and bacteria. Liu applied time-course snRNA-seq to *M. truncatula* symbiotic roots at 30 min, 6 h, and 24 h after Nod factor treatment [80]. Significant reprogramming of gene expression in the epidermis and cortex was observed as early as 30 mpi, with most of these changes restored at 6 h. A coexpression module enriched for known symbiotic nitrogen fixation genes was further explored and revealed the involvement of *MtFER* in rhizobial perception. The researchers demonstrated that *MtFER* can promote root growth and impact symbiotic and defense-related gene expression [80]. The most recent single-cell transcriptome dataset in *M. truncatula* from Pereira *et al.* [81] provides a detailed exploration of root nodule symbiosis (RNS) development. Covering four RNS stages (0, 24, 48, and 96 hpi), the study aimed to understand the cellular response to rhizobia infection focusing on the

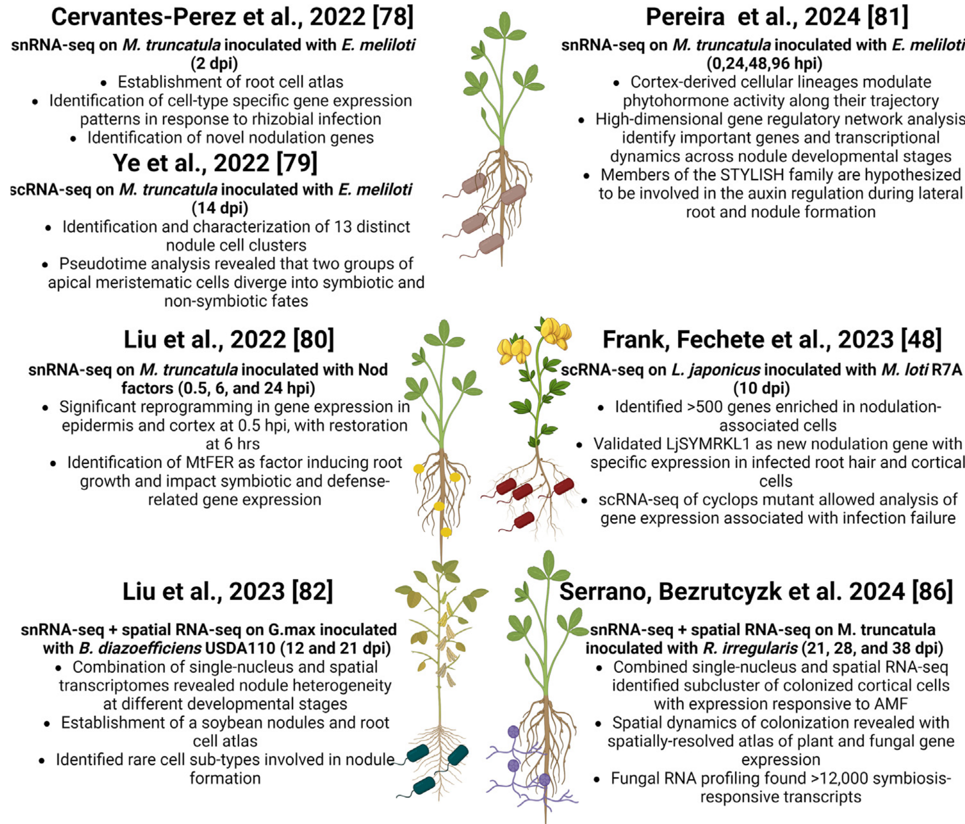

Figure 2. Recent transcriptomic discoveries in plant–microbe endosymbioses [48,78–82,86]. Summary of the key findings from recent literature analyzing plant–microbe endosymbiosis via single-cell (sc)/single-nucleus (sn) and spatial RNA-seq. Each publication is referenced using the author and publication date and is displayed next to a schematic of what symbiotic system was analyzed. Abbreviations: AMF, arbuscular mycorrhizal fungi; dpi, days post-inoculation; hpi, hours post-inoculation; mpi, minutes post-inoculation. Figure created with BioRender.com.

cortex and pericycle layers crucial for nodule formation. To overcome the limitation of rare cells responding to rhizobia infection, the authors integrated single-cell data from both wild-type and hypernodulating mutant plants. This approach allowed the authors to discover important genes and transcriptional dynamics across nodule development stages. For instance, it uncovered how MtHB1 suppresses auxin signaling genes during early cortex cell differentiation, activates auxin biosynthesis genes in developing nodule primordia, and manages cytokinin inactivation and degradation in distinct nodule compartments. Despite its thorough insights into nodule meristem

Table 2. sc/snRNA-seq symbiosis studies

Cell/nucleus number	Median genes per cell/nucleus	Refs
28 375 nuclei	1053	[78]
10 814 protoplasts	1620	[79]
26 712 nuclei	1018	[80]
25 024 protoplasts	1500	[48]
16 211 nuclei	Not reported, 36 131 total genes	[81]
26 712 nuclei	1342	[82]
16 890 nuclei	1120	[86]

formation from cortex cells, the study has limitations in capturing all cell lineages and developmental phases. Nonetheless, it lays the groundwork for future comparative analyses between lateral root and nodule development, utilizing the identified cell types and lineages for deeper exploration.

Two additional studies were conducted using legume species that form determinate nodules. Frank, Fechete, *et al.* used scRNA-seq of *Lotus japonicus* at 10 dpi to define root hair and cortical cell populations involved in rhizobium infection [48]. More than 500 genes with enriched expression in nodulation-associated cells were identified, providing a valuable resource for further analysis. The researchers were also able to validate a new nodulation gene, *LjSYMRKL1*, with expression specific to infected root hair and cortical cells. Last, scRNA-seq of protoplasted root susceptible zones of the *cyclops* mutant allowed the identification of gene expression associated with infection failure [48]. Liu *et al.* [82] combined snRNA-seq and spatial RNA-seq on nodules (12 and 21 dpi) in *Glycine max* as well as the root regions where nodules formed. While root cell types could be easily identified, the lack of marker genes for *G. max* nodules made it difficult to assign cell clusters. To tackle this issue, they utilized Stereo-Seq and tracked gene expression in nodules at the same developmental stage. Using histological features and deconvolution of spatial and snRNA-seq expression data, they classified most major cell types of the root and nodule [82].

Research on plant–pathogen interactions [5,10,83–85] demonstrated the utility of novel transcriptomic methodologies to analyze plant–fungal relationships. Single-nucleus and ST were applied for the first time to the AMF symbiosis between *M. truncatula* and *Rhizophagus irregularis* [86]. Serrano, Bezrutcyzk, *et al.* combined Chromium and Visium to construct a spatially resolved transcriptome map containing genes from both species at 28 dpi. Fungal nuclei were not captured by the Chromium platform. However, Visium did allow plant and fungal transcripts to be captured simultaneously, with over 12 000 fungal transcripts captured across the nine capture areas [86]. The resulting datasets present a novel transcriptomic resource for the arbuscular mycorrhizal symbiosis community; however, the limited resolution of the spatial technology did not allow cell-type-specific analyses of the fungal transcripts, as most voxels may contain different cell types. Spatial technologies with increased resolution, such as Stereo-Seq and Seq-Scope, also have the potential to capture fungal transcripts and represent great tools to disentangle the cell-type-specific transcriptomic signature of the arbuscular mycorrhizal symbiosis.

Limitations to transcriptomic investigation of symbiosis

Identifying cell types from transcriptome data relies on manual annotation of clusters using reference marker genes. For non-model plant, bacterial, and fungal species without an available curated database of marker genes, this can be a difficult and time-consuming task. This issue is exacerbated when profiling rare cell types, which require processing of large cell populations, adding to experimental costs. The capture of low-abundance cells engaged in early responses in root hair or cortical cells may demand increased sequencing depth, subsequently elevating the overall expense and length of analyses. Several groups are working on developing solutions that will allow enrichment of low-abundance cells [87], such as the development of ONE-SENSE [88] and DA-Seq [89].

scRNA-seq and spatial RNA-seq studies generate an immense amount of data. The need for standardized and accessible experimental workflows, data processing/analysis pipelines, and data deposition practices increases with the method's popularity. scRNA-seq datasets face significant challenges, necessitating stringent quality control, normalization to correct for biases and batch effects, and the use of dimensionality reduction techniques like PCA, UMAP, or tSNE, along with robust clustering algorithms to identify distinct cell populations. Differential

expression analysis and lineage inference using algorithms such as Monocle require careful parameter tuning to ensure accurate and valid results. Many researchers are publishing transcriptome data for the same species using similar methodologies, but without a public centralized database for symbiosis research with established experimental standards it is difficult to compare data across multiple studies. Recent efforts such as SpatialDB [90], a public web curation of spatially resolved transcriptome data, and the Plant Cell Atlas Consortium [91], a scientific framework focusing on building a single-cell multiomics atlas of developing model plants, are laying the groundwork, but standard repositories are for more specialized efforts.

Another major obstacle is that it is not currently possible to capture both plant and bacterial transcripts simultaneously in the same cells. Plant mRNA transcripts are relatively easy to separate from rRNAs because they are polyadenylated, but bacterial mRNAs are less abundant and less stable and lack polyadenylation [92]. Quantifying gene expression from bacterial symbionts using scRNA-seq requires very efficient counterselection against bacterial rRNAs, likely coupled with deep sequencing to detect bacterial mRNAs in an RNA pool dominated by plant transcripts. Furthermore, polyadenylation of bacterial mRNAs following rRNA depletion would be required to allow compatibility with standard scRNA-seq procedures, including the 10X Chromium protocols (https://www.10xgenomics.com). To capture bacterial single-cell data, bacterial cells would have to be physically separated from plant cells, as previously demonstrated in *A. thaliana* leaves [93], prior to the application of prokaryotic scRNA-seq techniques such as microSPLiT [94] and PETRI-seq [95], precluding simultaneous capture of information from both host and symbiont. Spatial transcriptomic methods have the same challenges with respect to the detection of microbial mRNAs but are able to capture polyadenylated fungal transcripts [86], and spatial metatranscriptomics (SmT) allows the capture of bacterial and fungal microbial signatures for community structure analysis via 16S, 18S, and ITS probes [84]. Spatial, hybridization-based methods should in principle be able to capture both plant and bacterial mRNAs but require the design of specific probes, necessitating prior knowledge of both host and symbiont target genes.

AMF contain multinucleate hyphae in a connected cytoplasmic space and have an extremely high number of nuclei in each cell, approximately two orders of magnitude higher than any fungal relatives [96,97]. Additionally, the diameter size of these nuclei can vary significantly between species and between life stages of the same species [97], which can complicate nucleus capture and filtering during snRNA-seq workflows. However, AMF currently remain more amenable than rhizobia to within-host single-cell analysis because of their polyadenylated mRNAs. Capturing single-cell rhizobial transcriptional signatures, together with those of their host plant cells, in ITs and nodules will have to await further technological developments, where a likely first step would rely on spatial, *in situ* hybridization-based technology (Table 1).

Concluding remarks and future perspectives

Plant–microbial endosymbioses have distinct biological characteristics that limit the power of traditional transcriptomic approaches. Novel technologies represent great potential for their application to studies of such symbiotic relationships as they enable cell-type- and morphological-feature-specific analyses of gene expression from both partners. Here, we provide an overview of the main single-cell and spatial methodologies that have emerged over recent years and speak to their advantages and disadvantages. Scientists have applied some of these sc/snRNA-seq or ST approaches in isolation or in unison to analyze the gene expression occurring between two species in either the plant–mycorrhizal or the legume–rhizobial symbiosis. This research has enriched our understanding of plant–microbe endosymbiosis, particularly the legume–rhizobial symbiosis, to which many groups have applied sc/snRNA-seq and

Outstanding questions

What open-access resources need to be created for a comprehensive single-cell, spatially resolved atlas of plant–microbe endosymbiosis to come to fruition?

What advances can be made in transcriptomic techniques to enable the simultaneous capture of transcripts from both prokaryotic and eukaryotic partners in symbiotic interactions, overcoming current challenges associated with low bacterial mRNA content and the lack of polyadenylation in bacteria?

What method development is needed to transcriptionally profile rhizobial and mycorrhizal co-infection in plants and reveal how these two symbionts coexist within the same plant?

How could trajectory inference analysis be adopted to model the developmental progress of colonization?

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identified new marker genes for symbiotic structures and processes as well as new symbiosis-specific candidate genes for functional characterization. For the arbuscular mycorrhizal symbiosis, pioneering work that combined snRNA-seq and spatial RNA-seq constructed a spatially resolved, high-resolution map of gene expression from both species and identified thousands of symbiosis-responsive fungal transcripts for the first time. However, these studies are limited by the current technologies. Future method development, particularly the development of a spatially resolved single-cell platform that allows concurrent prokaryotic and eukaryotic transcript capture, will have increased power to answer outstanding questions. As data are generated, the creation of a public, centralized database with standards for data collection and analysis that allows users to browse scRNA-seq and spatial RNA-seq data across species will prove critical to the effort of building a single-cell gene expression atlas for endosymbiosis (see Outstanding questions).

Acknowledgments

This study was supported by the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the US Department of Energy, Office of Science, Biological and Environmental Research Program, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US Department of Energy, by Independent Research Fund Denmark (grant agreement no. 1026-00032B), and by the project Molecular Mechanisms and Dynamics of Plant–Microbe Interactions at the Root–Soil Interface (InRoot), supported by Novo Nordisk Foundation grant no. NNF19SA0059362.

Declaration of interests

The authors declare no competing interests.

References

1. Seyfferth, C. *et al.* (2021) Advances and opportunities in single-cell transcriptomics for plant research. *Annu. Rev. Plant Biol.* 72, 847–866
2. Cervantes-Pérez, S.A. *et al.* (2022) Review: challenges and perspectives in applying single nuclei RNA-seq technology in plant biology. *Plant Sci.* 325, 111486
3. Denyer, T. and Timmermans, M.C.P. (2022) Crafting a blueprint for single-cell RNA sequencing. *Trends Plant Sci.* 27, 92–103
4. Mo, Y. and Jiao, Y. (2022) Advances and applications of single-cell omics technologies in plant research. *Plant J.* 110, 1551–1563
5. Zhu, J. *et al.* (2023) Understanding plant pathogen interactions using spatial and single-cell technologies. *Commun. Biol.* 6, 814
6. Yin, R. *et al.* (2023) Spatial transcriptomics drives a new era in plant research. *Plant J.* 116, 1571–1581
7. Oldroyd, G.E.D. (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* 11, 252–263
8. Roy, S. *et al.* (2020) Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. *Plant Cell* 32, 15–41
9. Lin, J. *et al.* (2020) No home without hormones: how plant hormones control legume nodule organogenesis. *Plant Commun.* 1, 100104
10. Kelly, S. *et al.* (2018) Distinct *Lotus japonicus* transcriptomic responses to a spectrum of bacteria ranging from symbiotic to pathogenic. *Front. Plant Sci.* 9, 1218
11. Breakspear, A. *et al.* (2014) The root hair "infectome" of *Medicago truncatula* uncovers changes in cell cycle genes and reveals a requirement for auxin signaling in rhizobial infection. *Plant Cell* 26, 4680–4701
12. Wang, W. *et al.* (2017) Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. *Mol. Plant* 10, 1147–1158
13. MacLean, A.M. *et al.* (2017) Plant signaling and metabolic pathways enabling arbuscular mycorrhizal symbiosis. *Plant Cell* 29, 2319–2335
14. Gaude, N. *et al.* (2012) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69, 510–528
15. Hogekamp, C. and Küster, H. (2013) A roadmap of cell-type specific gene expression during sequential stages of the arbuscular mycorrhiza symbiosis. *BMC Genomics* 14, 306
16. Kretzschmar, T. *et al.* (2012) A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature* 483, 341–344
17. Waters, M.T. *et al.* (2017) Strigolactone signaling and evolution. *Annu. Rev. Plant Biol.* 68, 291–322
18. Pimprikar, P. and Gutjahr, C. (2018) Transcriptional regulation of arbuscular mycorrhiza development. *Plant Cell Physiol.* 59, 678–695
19. Luginbuehl, L.H. and Oldroyd, G.E.D. (2017) Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. *Curr. Biol.* 27, R952–R963
20. Bapaume, L. and Reinhardt, D. (2012) How membranes shape plant symbioses: signaling and transport in nodulation and arbuscular mycorrhiza. *Front. Plant Sci.* 3, 223
21. Pumplin, N. and Harrison, M.J. (2009) Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. *Plant Physiol.* 151, 809–819
22. Kobae, Y. and Fujiwara, T. (2014) Earliest colonization events of *Rhizophagus irregularis* in rice roots occur preferentially in previously uncolonized cells. *Plant Cell Physiol.* 55, 1497–1510
23. Camps, C. *et al.* (2015) Combined genetic and transcriptomic analysis reveals three major signalling pathways activated by Myc-LCOs in *Medicago truncatula*. *New Phytol.* 208, 224–240
24. Bonneau, L. *et al.* (2013) Combined phosphate and nitrogen limitation generates a nutrient stress transcriptome favorable for arbuscular mycorrhizal symbiosis in *Medicago truncatula*. *New Phytol.* 199, 188–202
25. Hohnjec, N. *et al.* (2005) Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different glomus fungi provide insights into the genetic program activated during arbuscular mycorrhiza. *Plant Physiol.* 137, 1283–1301
26. Cope, K.R. *et al.* (2022) Physiological and transcriptomic response of *Medicago truncatula* to colonization by high- or low-benefit arbuscular mycorrhizal fungi. *Mycorrhiza* 32, 281–303
27. Apelt, F. *et al.* (2022) Shoot and root single cell sequencing reveals tissue- and daytime-specific transcriptome profiles. *Plant Physiol.* 188, 861–878

28. Chen, W. *et al.* (2021) Arbuscular mycorrhizal fungus alters root system architecture in *Camellia sinensis* L. as revealed by RNA-seq analysis. *Front. Plant Sci.* 12, 777357
29. Cervantes-Gámez, R.G. *et al.* (2016) Arbuscular mycorrhizal symbiosis-induced expression changes in *Solanum lycopersicum* leaves revealed by RNA-seq analysis. *Plant Mol. Biol. Report.* 34, 89–102
30. Mateus, I.D. *et al.* (2019) Dual RNA-seq reveals large-scale non-conserved genotype × genotype-specific genetic reprograming and molecular crosstalk in the mycorrhizal symbiosis. *ISME J.* 13, 1226–1238
31. Zouari, I. *et al.* (2014) From root to fruit: RNA-seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. *BMC Genomics* 15, 221
32. Handa, Y. *et al.* (2015) RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in *Lotus japonicus* and *Rhizophagus irregularis*. *Plant Cell Physiol.* 56, 1490–1511
33. Manley, B.F. *et al.* (2023) A highly contiguous genome assembly reveals sources of genomic novelty in the symbiotic fungus *Rhizophagus irregularis*. *G3 (Bethesda)* 13, jkad077
34. Dallaire, A. *et al.* (2021) Transcriptional activity and epigenetic regulation of transposable elements in the symbiotic fungus *Rhizophagus irregularis*. *Genome Res.* 31, 2290–2302
35. Yildirir, G. *et al.* (2022) Long reads and Hi-C sequencing illuminate the two-compartment genome of the model arbuscular mycorrhizal symbiont *Rhizophagus irregularis*. *New Phytol.* 233, 1097–1107
36. Beaudet, D. *et al.* (2018) Ultra-low input transcriptomics reveal the spore functional content and phylogenetic affiliations of poorly studied arbuscular mycorrhizal fungi. *DNA Res.* 25, 217–227
37. Zeng, T. *et al.* (2018) Host- and stage-dependent secretome of the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Plant J.* 94, 411–425
38. Keller-Pearson, M. *et al.* (2023) A dual transcriptomic approach reveals contrasting patterns of differential gene expression during drought in arbuscular mycorrhizal fungus and carrot. *Mol. Plant-Microbe Interact.* 36, 821–832
39. Chen, E.C. *et al.* (2018) Single nucleus sequencing reveals evidence of inter-nucleus recombination in arbuscular mycorrhizal fungi. *eLife* 7, e39813
40. Maeda, T. *et al.* (2018) Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*. *Commun. Biol.* 1, 87
41. Tisserant, E. *et al.* (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769
42. Lanfranco, L. and Bonfante, P. (2023) Lessons from arbuscular mycorrhizal fungal genomes. *Curr. Opin. Microbiol.* 75, 102357
43. Geurts, R. and Vleeshouwers, V.G.A.A. (2012) Mycorrhizal symbiosis: ancient signalling mechanisms co-opted. *Curr. Biol.* 22, R997–R999
44. Wang, D. *et al.* (2022) Innovation and appropriation in mycorrhizal and rhizobial symbioses. *Plant Cell* 34, 1573–1599
45. Genre, A. and Russo, G. (2016) Does a common pathway transduce symbiotic signals in plant–microbe interactions? *Front. Plant Sci.* 7, 96
46. Jovic, D. *et al.* (2022) Single-cell RNA sequencing technologies and applications: a brief overview. *Clin. Transl. Med.* 12, e694
47. Van Den Brink, S.C. *et al.* (2017) Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. *Nat. Methods* 14, 935–936
48. Frank, M. *et al.* (2023) Single-cell analysis identifies genes facilitating rhizobium infection in *Lotus japonicus*. *Nat. Commun.* 14, 7171
49. Denyer, T. *et al.* (2019) Spatiotemporal developmental trajectories in the *Arabidopsis* root revealed using high-throughput single-cell RNA sequencing. *Dev. Cell* 48, 840–852.e5
50. Zheng, D. *et al.* (2023) Recent progresses in plant single-cell transcriptomics. *Crop Des.* 2, 100041
51. Marx, V. (2021) Method of the year: spatially resolved transcriptomics. *Nat. Methods* 18, 9–14
52. Giacomello, S. (2021) A new era for plant science: spatial single-cell transcriptomics. *Curr. Opin. Plant Biol.* 60, 102041
53. Moses, L. and Pachter, L. (2022) Museum of spatial transcriptomics. *Nat. Methods* 19, 534–546
54. Chen, C. *et al.* (2023) Opportunities and challenges in the application of single-cell and spatial transcriptomics in plants. *Front. Plant Sci.* 14, 1185377
55. Cheng, M. *et al.* (2023) Spatially resolved transcriptomics: a comprehensive review of their technological advances, applications, and challenges. *J. Genet. Genomics* 50, 625–640
56. Ståhl, P.L. *et al.* (2016) Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 353, 78–82
57. Rodriques, S.G. *et al.* (2019) Slide-Seq: a scalable technology for measuring genome-wide expression at high spatial resolution. *Science* 363, 1463–1467
58. Stickels, R.R. *et al.* (2021) Highly sensitive spatial transcriptomics at near-cellular resolution with Slide-Seq V2. *Nat. Biotechnol.* 39, 313–319
59. Vickovic, S. *et al.* (2019) High-definition spatial transcriptomics for *in situ* tissue profiling. *Nat. Methods* 16, 987–990
60. Liu, Y. *et al.* (2020) High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue. *Cell* 183, 1665–1681.e18
61. Cho, C.-S. *et al.* (2021) Microscopic examination of spatial transcriptome using Seq-Scope. *Cell* 184, 3559–3572.e22
62. Chen, A. *et al.* (2022) Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* 185, 1777–1792.e21
63. Yue, L. *et al.* (2023) A guidebook of spatial transcriptomic technologies, data resources and analysis approaches. *Comput. Struct. Biotechnol. J.* 21, 940–955
64. Chen, K.H. *et al.* (2015) Spatially resolved, highly multiplexed RNA profiling in single cells. *Science* 348, aaa6090
65. Shah, S. *et al.* (2016) *In situ* transcription profiling of single cells reveals spatial organization of cells in the mouse hippocampus. *Neuron* 92, 342–357
66. Eng, C.-H.L. *et al.* (2019) Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+. *Nature* 568, 235–239
67. Groiss, S. *et al.* (2021) Highly resolved spatial transcriptomics for detection of rare events in cells. *bioRxiv*, Published online October 12, 2021. https://doi.org/10.1101/2021.10.11.463936
68. Merritt, C.R. *et al.* (2020) Multiplex digital spatial profiling of proteins and RNA in fixed tissue. *Nat. Biotechnol.* 38, 586–599
69. Goh, J.J.L. *et al.* (2020) Highly specific multiplexed RNA imaging in tissues with Split-FISH. *Nat. Methods* 17, 689–693
70. Borm, L.E. *et al.* (2022) Scalable *in situ* single-cell profiling by electrophoretic capture of mRNA using EEL FISH. *Nat. Biotechnol.* 41, 222–231
71. Nobori, T. *et al.* (2023) Multiplexed single-cell 3D spatial gene expression analysis in plant tissue using PHYTOMap. *Nat. Plants* 9, 1026–1033
72. Femino, A.M. *et al.* (1998) Visualization of single RNA transcripts *in situ*. *Science* 280, 585–590
73. Jean-Baptiste, K. *et al.* (2019) Dynamics of gene expression in single root cells of *Arabidopsis thaliana*. *Plant Cell* 31, 993–1011
74. Ryu, K.H. *et al.* (2019) Single-cell RNA sequencing resolves molecular relationships among individual plant cells. *Plant Physiol.* 179, 1444–1456
75. Shulse, C.N. *et al.* (2019) High-throughput single-cell transcriptome profiling of plant cell types. *Cell Rep.* 27, 2241–2247.e4
76. Zhang, T.-Q. *et al.* (2019) A single-cell RNA sequencing profiles the developmental landscape of *Arabidopsis* root. *Mol. Plant* 12, 648–660
77. Shahan, R. *et al.* (2022) A single-cell *Arabidopsis* root atlas reveals developmental trajectories in wild-type and cell identity mutants. *Dev. Cell* 57, 543–560.e9
78. Cervantes-Pérez, S.A. *et al.* (2022) Cell-specific pathways recruited for symbiotic nodulation in the *Medicago truncatula* legume. *Mol. Plant* 15, 1868–1888
79. Ye, Q. *et al.* (2022) Differentiation trajectories and biofunctions of symbiotic and un-symbiotic fate cells in root nodules of *Medicago truncatula*. *Mol. Plant* 15, 1852–1867
80. Liu, Z. *et al.* (2023) Single-nucleus transcriptomes reveal spatiotemporal symbiotic perception and early response in *Medicago*. *Nat. Plants* 9, 1734–1748
81. Periera, W. *et al.* (2024) The single-cell transcriptome program of nodule development cellular lineages in *Medicago truncatula*. *Cell Rep.* 43, 113747

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82. Liu, Z. *et al.* (2023) Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation. *Nat. Plants* 9, 515–524
83. Giolai, M. *et al.* (2019) Spatially resolved transcriptomics reveals plant host responses to pathogens. *Plant Methods* 15, 114
84. Saarenpää, S. *et al.* (2023) Spatial metatranscriptomics resolves host–bacteria–fungi interactomes. *Nat. Biotechnol.*, Published online November 20, 2023. https://doi.org/10.1038/s41587-023-01979-2
85. Tang, B. *et al.* (2023) Cell-type-specific responses to fungal infection in plants revealed by single-cell transcriptomics. *Cell Host Microbe* 31, 1732–1747.e5
86. Serrano, K. *et al.* (2024) Spatial co-transcriptomics reveals discrete stages of the arbuscular mycorrhizal symbiosis. *Nat. Plants* 10, 673–688
87. Nguyen, A. *et al.* (2018) Single cell RNA sequencing of rare immune cell populations. *Front. Immunol.* 9, 1553
88. Mair, F. *et al.* (2020) A targeted multi-omic analysis approach measures protein expression and low-abundance transcripts on the single-cell level. *Cell Rep.* 31, 107499
89. Zhao, J. *et al.* (2021) Detection of differentially abundant cell subpopulations in scRNA-seq data. *Proc. Natl. Acad. Sci. U. S. A.* 118, e20093118
90. Fan, Z. *et al.* (2019) SpatialDB: a database for spatially resolved transcriptomes. *Nucleic Acids Res.* 48, D233–D237
91. Plant Cell Atlas Consortium *et al.* (2021) Vision, challenges and opportunities for a Plant Cell Atlas. *eLife* 10, e66877
92. Sarkar, N. (1997) Polyadenylation of mRNA in prokaryotes. *Annu. Rev. Biochem.* 66, 173–197
93. Nobori, T. *et al.* (2018) Transcriptome landscape of a bacterial pathogen under plant immunity. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3055–E3064
94. Kuchina, A. *et al.* (2021) Microbial single-cell RNA sequencing by split-pool barcoding. *Science* 371, eaba5257
95. Blattman, S.B. *et al.* (2020) Prokaryotic single-cell RNA sequencing by *in situ* combinatorial indexing. *Nat. Microbiol.* 5, 1192–1201
96. Sperschneider, J. *et al.* (2023) Arbuscular mycorrhizal fungi heterokaryons have two nuclear populations with distinct roles in host–plant interactions. *Nat. Microbiol.* 8, 2142–2153
97. Kokkoris, V. *et al.* (2020) Nuclear dynamics in the arbuscular mycorrhizal fungi. *Trends Plant Sci.* 25, 765–778