Trends in Plant Science

CellPress

Forum

Plant–microbiome dynamics through spatial metatranscriptomics and network biology

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Climate change threatens global agriculture, impacting plant health and crop yield, while plant microbiomes offer potential solutions to enhance resilience. In this forum, we discuss the prospects of single cell multiome and network science in understanding intricate plant–microbe interactions, providing insights for sustainable agriculture and improved crop productivity for global food security.

Enhancing plant resilience in a changing climate

Climate change has far-reaching consequences on global food security, heightening plant vulnerability to pests and pathogens, and resulting in an estimated 30% annual crop loss worldwide [1], raising serious critical questions. How do different plant species cope with the rapidly changing climate worldwide? Do they exhibit the adaptability and resilience to survive the changing habitats? Does the plant microbiome have a significant role in enhancing resilience against environmental stresses? To address these questions, among others, it is crucial to understand the complex relationship between plants and their associated microbes. As such, plant-associated bacteria regulate physiological and molecular changes in plants, substantially contributing to nutrient cycling, disease resistance, and stress tolerance, thereby exerting a profound influence on plant growth, reproduction, and overall yield [1]. Hence, the exploration of such biological alternatives provides promising solutions for sustainable agriculture, complementing conventional chemical-based pest and disease control strategies [1,2]. However, there remains a gap in deciphering the regulatory mechanisms and key participants involved in plant-beneficial microbe interactions within specific microenvironments, utilizing both conventional high-throughput sequencing technologies and computational tools and pipelines [3]. Here, we discuss the current state of, and prospects for, plant microbiome research, highlighting cell type-specific responses in interpreting sophisticated dynamics of plant–microbe interactions.

Integrative approaches of single cell multiomics for understanding plant–microbe dynamics

The advent of single cell sequencing has markedly enhanced the capacity to analyze intricate molecular signatures at individual cell levels under diverse biotic and abiotic stresses [1]. Such technology has expanded its scope to exploring shared molecular signatures across distinct cell populations, providing crucial details of their spatial organization within tissues and microenvironments [4]. In investigations in humans and other mammals, single cell biology has extensively examined cell heterogeneity shaped by physiological circumstances or disease progression, utilizing the capabilities of microfluidic technology. This approach has been further extended to uncover functional variability within human microbiomes through integrated meta-genomic and meta-transcriptomic methods [5]. In line with advances in plant–microbe interaction research, single cell and spatial technologies have significantly enriched our understanding of cellular heterogeneity [6]. Techniques, such as single cell multiome sequencing and MERFISH, have elucidated gene regulatory networks at a single cell resolution in arabidopsis (*Arabidopsis thaliana*) [7] (Figure 1). This single cell resolution has been enhanced through spatial metatranscriptomics (SmT), which captures both prokaryotic and eukaryotic microbial identities along with host transcriptomic signals, thereby unveiling microbial organization and host immune responses (Figure 1), the details of which are covered in a recent review by Ntekas and De Vlaminck [8].

Furthermore, live cell imaging and single cell sequencing were used to identify spatial heterogeneity in the expression of intracellular immune receptor NLR genes and revealed the significance of the glucosinolate pathway and the key player, MYB122, in fungal disease resistance [9]. In addition, technologies, such as 10x Genomics Chromium and Stereo-seq, provide high-resolution gene expression maps, while SMART-seq identifies defense mechanisms that can aid crop improvement (reviewed by Zhu and colleagues [7)] (Figure 1).

Using the aforementioned technologies, a multiomics model can be developed to influence plant–microbe interactions, thereby enabling targeted crop improvement based on single cell and spatial transcriptomics data. For instance, utilizing a targeted NLR model system alongside spatial transcriptomics techniques, such as Stereo-seq and GaST-seq, can map molecular dynamics in space and time, enhancing understanding of pathogen resistance mechanisms. Multiomics analyses, including metagenomics, proteomics, and metabolomics, can build predictive network models identifying key regulatory elements and pathways involved in plant defense. With the structural and functional modulation of predicted regulators, a eukaryotic pathogen-free crop plant can be developed by manipulating the plant–microbe interactions. This high-throughput multiomics approach offers a powerful tool to mitigate environmental stresses, ensuring food security under

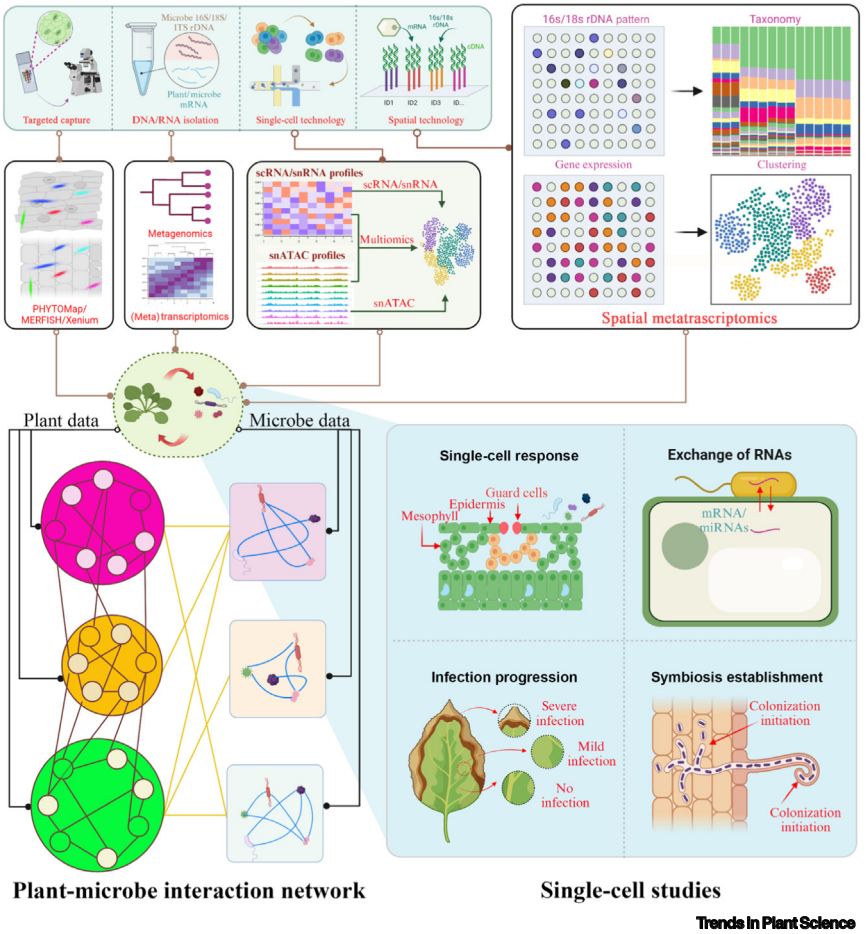

Figure 1. Construction of a single cell and spatial plant–microbe interaction module. Advances in sequencing and visualization technologies have revolutionized the capture of plant and microbe information, providing varied insights into plant–microbe interactions. Techniques, such as PHYTOMap, MERFISH, and Xenium, facilitate the targeted capture of mRNA and enable spatial visualization of cellular gene expression. Genome and transcriptomics sequencing provides comprehensive DNA and RNA profiles from whole tissues. Additionally, cell or nuclei isolation followed by processing through single cell platforms allows for the detailed analysis of mRNA or chromatin accessibility at a single cell resolution. The cutting-edge field of spatial transcriptomics, particularly the novel spatial metatranscriptomics (SmT), enables researchers to concurrently map cellular transcriptomes and bacterial identities with near single cell precision. All the aforementioned technologies have been used in plant–microbe interaction studies. By integrating both plant and microbial readouts, it becomes possible to construct detailed plant gene regulatory networks and microbe–microbe interaction networks. These networks are further integrated to form a sophisticated spatial plant–microbe interaction model. This model facilitates further studies, such as identifying plant cell type-specific responses to microbes, revealing correlations during infection progression, cross-kingdom RNA exchanges, and symbiosis establishment. Abbreviations: ATAC, assay for transposase-accessible chromatin; ITS, internal transcribed spacer; rDNA, ribosomal DNA; scRNA, single cell RNA; snRNA, single nucleus RNA.

changing climatic conditions. The continuous refinement of single cell-sequencing technologies promises unprecedented insights into cellular diversity, molecular dynamics, and the functional landscapes of host and microbial ecosystems.

Advancing single cell metatranscriptomics with network biology

Network biology has revolutionized our knowledge of complex biological systems by integrating multiple 'omics layers to reveal intricate interactions within and between species. Holo-omics integrates multiomics data from the host and its microbiota. It can effectively elucidate functional plant–microbial relationships by exploring meta-genomics, meta-transcriptomics, meta-proteomics, and meta-metabolomics data sets to provide a holistic view of their interactions. A central aspect of holo-omics is network reconstruction, integrating data from different 'omics layers to form a coherent model [10] (Figure 2). Metatranscriptomics emerges as a cutting-edge approach for reconstructing networks and deciphering the complex microbial interactions and gene expression patterns. Tools, including HUMAnN2, MetaTrans, and SAMSA, can be used to build co-occurrence and metabolic networks via taxonomic and functional profiling, unraveling key regulatory nodes and their gene-level interactions (Figure 2). Integrating single cell metatranscriptomics with network biology using models, including weighted gene co-expression network analysis (WGCNA) and high-dimensional (hd)-WGCNA, allows the construction of cell type-specific interaction networks, identifying modules of highly co-expressed genes and key hub and bottleneck nodes [11] (Figure 2). For instance, the spatial multiomics approach allows the capture of major immune active cells in mesophyll and epidermal cell clusters in response to effector-triggered immunity (ETI)-triggering bacterial and fungal strains. Interestingly, the subclustering of the immune-activated cells facilitates characterization of the distinct immune gene-enriched phloem companion cells, indicating that not all mesophyll cells participate in the defense response [9,12]. This presents an opportunity to differentiate between immune-specific modules and other mesophyll-related transcriptomes. Additionally, incorporating metatranscriptomic inputs with protein–protein interaction (PPI) data further improves cellular processes study by identifying cell type-specific PPIs, particularly in plant–pathogen interactions.

This strategy can ascertain the cell-specific responses to virulent infections, such as in guard cells, companion cells, and mesophyll cells. Moreover, the PINNACLE approach, a flexible geometric deep learning model, constructs cell type-specific protein interaction networks to examine interactions across tissues and cell types, characterizing their roles in cellular function and communication [13] (Figure 2). Network analysis techniques can decipher topological properties, including node degree, clustering coefficient, various centralities, and bipartite betweenness centrality (BiBC), to identify key regulatory nodes. High-degree hubs control biological pathways, while BiBC-identified bottleneck nodes act as bridges within the network. Detection of the cell-specific targeted 'party' and 'date' hubs and/or bottlenecks can reveal the diverse regulatory players in networks and nutrient flow utilizing mechanisms by virulent and beneficial microbes through interactions and communications across cells (Figure 2).

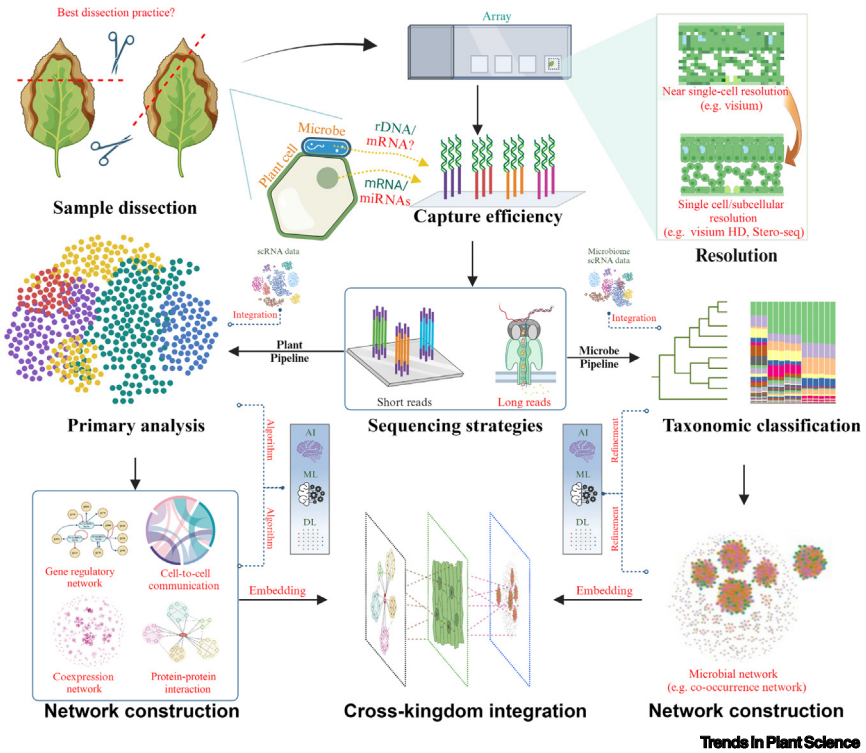

Figure 2. Opportunities and challenges in spatial plant–microbe interactions. The intricacies and potential of spatial plant–microbe interaction studies are illustrated alongside benchwork and data analysis procedures. Starting with sample collection, optimal dissection practices are vital to recover extensive microbial and plant cell type-specific responses. Advances are expected in the spatial resolution of microwell arrays, aiming to facilitate the high-fidelity capture of single cell profiles. Equally challenging and opportunistic is the design of distinct molecular probes for capturing a broader spectrum of plant and microbial genomics and transcriptomic features, including plant miRNAs and microbial mRNA. Follow-up sequencing strategies, such as short and long-read sequencing, also impact the comprehensive extraction of RNA or DNA information from both kingdoms. Post sequencing, the obtained readout is further bifurcated; reads aligning with the plant genome are processed through a spatial transcriptomic analysis pipeline, whereas microbial reads follow microbial amplicon sequencing analysis pipelines. Within the plant pipeline, programs using arbitrary cutoffs often result in losing cells with low mRNA contents, requiring better algorithms to refine cell separation. Subsequent inference of gene regulatory networks also relies on powerful computational tools capable of coping with sparse and noisy data presented by individual cell populations. Concurrently, microbial reads assigned to the microbe pipeline are refined for taxonomic classification and spatial contextualization, benefiting from current strides in artificial intelligence/machine learning/deep learning (AI/ML/DL). Microbial network construction and analysis further elucidate microbe–microbe interactions and screen top genera in each spatial context. Ultimately, inferred diverse networks, including co-expression, regulatory, and protein–protein interaction networks, from both plants and microbes are spatially integrated. For microbial data, future enhancements in capture assays are anticipated to enable spatial metatranscriptomic studies, which could be further integrated with metagenomics data to achieve microbial gene regulatory network construction. Additionally, AI/ML/DL-driven modules hold promise for augmenting various aspects of the data analysis pipeline in both kingdoms. Abbreviation: rDNA, ribosomal DNA; scRNA, single cell RNA.

Furthermore, transkingdom network analysis (TkNA) can elucidate host–microbiota interactions by reconstructing networks that capture statistical relationships across different 'omics layers. TkNA incorporates single cell transcriptomics to infer cell types and detect key regulatory nodes, revealing interconnections of biological processes across species and providing a holistic view of host–microbiota interplay [14]. Cell–cell communication within these networks is critical for finding plant–microbe interactions. For example, beneficial bacteria, such as auxin-producing microbes, promote plant growth by communicating with root cells and other plant tissues. Identifying nodes that facilitate communication can offer insights into the establishment and maintenance of beneficial interactions [12] (Figure 2). This information is crucial for designing strategies to enhance plant fitness through microbiome manipulation. Additionally, integrating genome-scale metabolic models (GEMs) with multiomics data enhances the conception of microbial community metabolism. Condition-specific community GEMs (CoCo-GEMs) encompass metatranscriptomic data to infer metabolic networks under specific conditions, providing a scalable framework for exploring biological hypotheses in microbial systems [15] (Figure 2). Studying changes in microbes and building regulatory networks within them can reveal mechanisms of virulence or beneficial interactions. The ability to model these networks on a genome scale is essential for capturing metabolic interactions within microbial communities

and between microbes and their hosts. Visualization of these complex networks is also important for interpreting and communicating findings. Tools, such as Cytoscape, can create detailed network graphs, highlighting key regulatory nodes and their interactions. Dot plots can visualize the degree distribution and other node properties, while the structure and function of network can illustrate the expression levels or the abundance of top regulatory nodes (Figure 2). A deepened understanding of these interactions has the potential to improve crop health and productivity through targeted agricultural strategies and innovative biotechnologies.

Future prospects and challenges

Interpretation of plant–microbe interactions has been considerably improved by the latest developments in sequencing and visualization technologies, such as PHYTOMap, MERFISH, Xenium, and SmT. Here, we propose a model for the integration of technologies on the same platform, applying different 'omics and network biology methods. Techniques, including PHYTOMap, MERFISH, and Xenium, enable targeted mRNA capture and spatial gene expression visualization (Figure 1). Genome and transcriptomics sequencing provides comprehensive DNA and RNA profiles, while single cell platforms allow detailed mRNA and chromatin analysis. SmT permits precise mapping of cellular transcriptomes and bacterial identities. Integrating these technologies aids the construction of multifaceted plant gene regulatory and microbe–microbe interaction networks, forming a refined spatial plant–microbe interaction model. A promising new approach that combines single cell transcriptomics with PPI data using PINNACLE can reveal the influence of microbes on plant cells at a molecular level, pinpointing critical regulatory points and clarifying the response mechanism of different cells. This model facilitates studies of plant cell type-specific responses, infection progression, RNA exchanges, and symbiosis establishment across kingdoms. Mapping cell-specific protein networks can identify key hubs controlling pathways and bottlenecks linking parts of the network (Figure 2). The interaction map can ultimately be used to help form spatial models that can decipher interkingdom interaction details at the cell-specific level. Predicting different interactions between pathogens and beneficial microbes can highlight disease mechanisms and positive effects on plant growth (Figure 1).

The construction of spatial plant–microbe interaction maps presents challenges and opportunities, requiring thorough benchwork and data analysis. Sample collection techniques are crucial for capturing comprehensive microbial and plant cell-specific responses. Advances in microwell arrays enhance single cell profiling resolution, but the thinness of plant leaves complicates cryo-sectioning. Longitudinal or cross-sectional approaches significantly impact the recovery of specific cell types, and optimization in optimal cutting temperature (OCT) embedding and tissue permeabilization is critical for diverse plant species. While technologies to identify spatiotemporal cell type-specific noncoding RNA signatures are still developing, recent advances, including VASA-seq and slide-tags, enable broader RNA or DNA capture. Designing molecular probes to capture diverse plant and microbial genomics, including miRNAs and mRNA, minimizes plant rRNA contamination and improves capture efficiency. Short and long-read sequencing strategies are vital for extracting RNA or DNA from both kingdoms. Metatranscriptomics is essential for investigating microbial functions and metabolism, identifying differentially expressed genes, and predicting phenotypic changes among microbes. Expanding random primers to capture various RNAs could enhance chip design. Refining post-sequencing data analysis and using artificial intelligence (AI), machine learning (ML), and/or deep learning (DL) can infer host regulatory networks and improve microbial classification. Integrating plant and microbial networks offers insights into spatial interactions, with future capture assay enhancements anticipated to enable detailed spatial metatranscriptomic studies (Figure 2).

Concluding remarks

In conclusion, our understanding of plant–microbiome dynamics has evolved due to transformative single cell-sequencing technologies. Such technologies could enable researchers to fully detangle the complex plant–microorganism relationships. Learnings from single cell sequencing can drive sustainable practices with fewer environmental impacts to address climate change challenges in agriculture.

Acknowledgments

This work was supported by United States National Science Foundation awards 2038872, 2318746, and 2418230 to M.S.M. We apologize to those authors whose original work we were unable to cite because of space limitations.

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https://doi.org/10.1016/j.tplants.2024.07.007

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