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Advances in single-cell and spatial omics for studying symbiotic nitrogen fixation: comparative cellular and evolutionary perspectives

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Abstract

Single-cell and spatial transcriptomics have revolutionized studies of symbiotic nitrogen fixation by resolving cellular heterogeneity, spatial gene-expression, and regulatory dynamics within root nodules. Recent investigations in model legumes have revealed conserved and species-specific programs controlling immune recognition, nodule development, and nitrogen-fixation metabolism. Integrating these datasets with single-cell epigenomic profiles, such as chromatin accessibility and three-dimensional genome architecture, provides new insight into epigenetic mechanisms that regulate key symbiotic genes. Comparative single-cell analyses across legumes and non-legumes elucidate phenotypic diversity and core regulatory networks of symbiotic nitrogen fixation at the cellular level, offering critical frameworks for engineering this process in non-legume crops.

Keywords: Comparative multi-omics, Nodulation-based nitrogen fixation, Single-cell transcriptomics, Spatial transcriptomics

Introduction

Symbiotic nitrogen fixation represents a mutually advantageous symbiotic relationship established via precise mutual recognition and coordination between plants and nitrogen-fixing bacteria [1]. This process efficiently transforms inert atmospheric nitrogen (N_2) into plant-available ammonia (NH_3), thereby playing a pivotal role in maintaining ecosystem stability and enhancing sustainable agriculture [2]. Since the first descriptions of nitrogen-fixing nodulation symbioses with rhizobia (in 1888) and *Frankia* (in 1895) [3, 4], the reorganization of the phylogenetic tree of angiosperms has demonstrated that plant species capable of forming nitrogen-fixing nodulation symbioses are confined to the orders Fabales, Fagales, Cucurbitales, and Rosales, which are collectively known as the nitrogen-fixing clade (NFC) [5, 6]. Only 10 of the 28 plant families within the NFC

contain nodulating taxa, and these nodule-forming families do not constitute a monophyletic group. Furthermore, the majority of the genera within 9 of these 10 families are incapable of forming nitrogen-fixing nodulation symbioses [7]. Legumes (Fabales) and the non-legume *Parasponia* (Rosales) engage in nodulation with rhizobia, besides species of the actinobacterial genus *Frankia* establish root nodule symbioses with actinorhizal plants from 8 different plant families across the orders Fagales, Cucurbitales, and Rosales [8, 9]. Among these, nitrogen fixation mediated by root nodules is considered the most efficient.

The formation of root nodules constitutes the central process of symbiotic nitrogen fixation, encompassing rhizobial infection, infection thread formation, symbiotic cell differentiation, and the establishment of mature symbiotic structures [10, 11]. Based on their developmental characteristics, rhizobial nodules can be classified into determinate nodules, in which meristematic activity ceases after initiation (e.g. those formed on the roots of *Phaseolus vulgaris*, *Lotus japonicus*, and *Glycine max*), and indeterminate nodules, which have persistent meristematic activity (e.g. those formed on *Medicago truncatula*, *Pisum sativum*, and *Trifolium* spp. roots). Infection modes also vary, including intercellular "crack" entry (e.g. in *Arachis hypogaea* roots) and intracellular infection thread formation via the root hair pathway. Additional classification criteria include the presence or absence of symbiosomes (individual or clustered bacteroids enclosed by a plant membrane-derived symbiosome membrane) and fixation threads (defined as modified infection threads that ramify within infected cells and harbor bacteroids during nitrogen fixation) [12, 13]. Nodulation involves complex signaling pathways associated with plant immunity, hormonal regulation, and lateral root development [10, 14–17]. Each stage entails distinct cell types and the intricate regulation of signal pathways. Several core genes have been experimentally validated to regulate nodulation and nitrogen fixation, including the flavonoid biosynthesis gene *MtCHS* [18], the central transcription factor *NIN* [19], immune-associated R genes [20], *MtCLEs* involved in the autoregulation of nodulation (AON) pathway [21, 22], the cell surface receptor kinases *MtLICK1/2* [23], and factors associated with the acquisition of *SHR-SCR* stem cell identity in plant cortical cells [24]. Over the past few decades, traditional transcriptome approaches at the whole-organism, tissue-specific, or cell-population-level transcriptome approaches, such as microdissection, have enabled the identification of key genes involved in symbiotic nitrogen fixation and nodule development [25–28]. However, these methods struggle to accurately distinguish among heterogeneous cell types within a tissue and fail to effectively uncover the precise spatial distribution of gene expression across such cells, thereby significantly limiting in-depth investigations into root nodule cell differentiation and the mechanisms of symbiotic nitrogen fixation.

Dissecting nitrogen fixation through single-cell technologies

In recent years, the rapid development and uptake of single-cell and spatial transcriptomics have offered novel methodological frameworks to address these challenges [29, 30]. Single-cell transcriptomics technologies with platforms such as 10 × Genomics Chromium and STAMP (Single-cell transcriptomics analysis and multimodal profiling through imaging) enable the analysis of cellular heterogeneity and functional states at single-cell resolution. Spatial transcriptomics platforms (10 × Visium, and Stereo-seq) map gene expression

spatially while preserving tissue structure and revealing specific patterns and domains. These technologies have been extensively applied in animal and biomedical research, identifying numerous key genes and therapeutic targets for a wide range of human diseases [31–34]. In addition, pan-single-cell transcriptome (PanSci) has also been applied to study cell-population dynamics [35]. Due to technical limitations, plant research has significantly lagged. Single-cell transcriptomics in plants can be categorized into two distinct types: single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq). Given the low yield of single cells extracted from plants, snRNA-seq has emerged as the predominant method. These technologies have enabled the construction of comprehensive multispecies single-cell expression atlases, including those for various plants and tissues, and have addressed various biological questions (Fig. 1A) [36–53]. Single-cell research in plants is growing steadily (Fig. 1B), such atlases have proven instrumental in plant developmental biology research, for example, in root morphogenesis and leaf senescence dynamics in *Arabidopsis thaliana*, endosperm differentiation in *Zea mays*, and the establishment of *Oryza sativa* embryo development trajectories [38, 50, 52, 53]. Further integration with single-cell ATAC sequencing (scATAC-seq) technology can reveal the coupling mechanism between chromatin accessibility dynamics and gene expression regulation, offering a multidimensional perspective for elucidating the epigenetic basis of cell heterogeneity [51, 54, 55].

Besides, single-cell technology offers a powerful strategy to overcome the challenges posed by cellular heterogeneity in studies related to nodule development and nitrogen fixation. Moreover, recent studies have demonstrated the feasibility and potential of single-cell techniques for investigating these processes. Notably, the mechanism of nodule

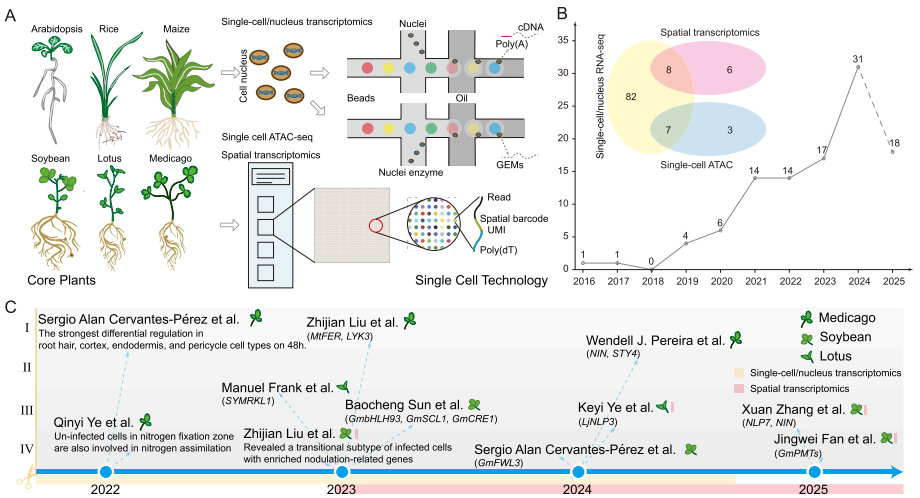

Fig. 1 Advances in plant single-cell omics and applications to nitrogen-fixing species. **A** Representative plant species for single-cell technology application and current single-cell solutions. **B** Annual publication counts and the number of studies performing multi-omics integration. Note: data obtained from Dimensions (https://www.dimensions.ai/) using the following research terms for plants: (single-cell transcriptomics and plants) or (single-nuclei transcriptomics and plants) or (spatiotemporal spatial transcriptomics and plants) or (single-cell ATAC and plants). **C** Advances in single-cell/nucleus and spatial transcriptome analyses of nodulating nitrogen-fixing plants. Note: The orange segments represent studies on single-cell transcriptomes, while the pink segments denote studies on spatial transcriptomes. Roman numerals illustrate the four key stages of nodule development: I. root hairs undergo curling; II. formation of the nodule primordium; III. small root nodule; IV. nodule maturation and the onset of nitrogen fixation

organogenesis for the establishment of symbiotic nitrogen fixation exhibits significant homology with that of lateral root development [56]. By employing single-cell multi-omics technology to analyze the spatiotemporal characteristics of cell lineage differentiation, hormone signal transduction, and the activation of key transcription factors during lateral root primordia formation, critical paradigms can be established to elucidate the reprogramming mechanism of host cells in the symbiotic nitrogen fixation system [57, 58]. These methodological advances not only extend the application boundaries of plant single-cell technology in non-model species but also establish a technical foundation for dissecting the dynamic regulatory network of cell-microbe interactions in this complex biological process.

Resolving root nodule cell diversity with single-cell transcriptomics

The construction of single-cell atlases for root nodules and the analysis of their regulatory networks have significantly lagged that of other plant organ systems owing to technical challenges, including the highly complex cellular heterogeneity, separation efficiency influenced by cell wall lignification, insufficient optimization of tissue-specific enzymatic digestion conditions, and interference from intracellular rhizobia. However, in recent years, single-cell transcriptome technology has made significant progress in the study of root nodules in model leguminous plants, such as *M. truncatula*, *G. max*, and *L. japonicus*, revealing the heterogeneity of cell types in root nodules of different species and differences in their gene expression (Fig. 1C).

In *M. truncatula*, single-cell transcriptome studies on the root nodule development process have provided novel insights into the symbiotic relationship between leguminous plants and rhizobia. These studies elucidated the dynamic regulatory networks governing the three key stages of root nodule development: (i) early immune recognition, (ii) cell division and organogenesis, and (iii) the mature nitrogen-fixing stage. Single-cell atlases of roots inoculated for 48 h, determined through snRNA-seq, revealed that root hairs and cortex cells exhibit significant differential regulatory features during the early symbiotic response. Key functional modules, such as nodulation factor (NF) signaling pathways, infection-related genes, plant hormones, and signal peptide molecules, displayed cell-type-specific expression patterns [59]. Time-series snRNA-seq of roots treated with Nod factors for 0.5, 6, and 24 h revealed finely tuned regulation of plant defense responses, in which defense-related genes are transiently activated at 0.5 h and rapidly downregulated thereafter. These studies also led to the classification of epidermal cells into two subcellular subtypes (subclusters 1–0 and 1–1), with NFs inducing distinct responses among these subclusters. In infected epidermal cells, *MtFER* and *MtLYK3* displayed a coordinated response pattern, and co-localized in a co-expression module rich in nodulation genes, suggesting that *MtLYK3*-mediated *MtFER* phosphorylation may serve as a hub for coordinating the expression of key genes involved in development, immunity, and symbiosis [60]. Notably, a recent study mapped, for the first time, the complete differentiation trajectory of *M. truncatula* nodule cells using snRNA-seq, revealing a stage-specific expression pattern of the classical symbiosis gene *NIN* in infected cells. *NIN* regulates hormone levels in different cortical cell types through its downstream target *STY4*, thereby orchestrating nodule development [61]. Regarding symbiosis, scRNA-seq analysis was first applied to *M. truncatula*, and

its results challenged the traditional understanding of the process and uncovered a considerable functional division of labor in nitrogen fixation metabolism: (i) infected cells specialize in assimilating atmospheric nitrogen into glutamine, whereas (ii) uninfected cells are responsible for subsequent conversion into asparagine. This finding refined the theoretical model that suggested that "nitrogen-fixing cells independently complete the entire metabolic process" [62, 63].

Recent studies performing the first scRNA-seq analyses in *L. japonicus* revealed that specific subpopulations of cells in the epidermis and cortex display distinct responses to rhizobial infection. Additionally, the protein SYMRKL1 (whose extracellular domain is highly similar to that of the symbiotic receptor SYMRK) has been identified as a critical regulatory factor for the proper formation of infection threads [64]. These studies have successfully resolved cell-specific processes that are challenging to discern using traditional bulk tissue expression analyses, thereby uncovering previously unrecognized transcriptional regulatory modules involved in symbiotic interactions. Related studies uncovered considerable differences in the nitrogen metabolism mechanisms in *G. max* and *M. truncatula* nodules. Unlike that of *M. truncatula,* which utilizes amide-dependent pathways, the nitrogen fixation metabolism of *G. max* nodules relies on ureide-based pathways. In the *G. max* symbiotic system, nitrogen metabolism exhibits a precise spatial division of labor, and infected cells specialize in synthesizing uric acid (UA), which is subsequently transported to the inner cortex of the nodule and converted into allantoic acid (Aln) by uricase. Aln and residual UA are further transported to the nodule pericycle, where they are metabolized into allantoic acid (Alc), by the enzyme allantoinase, and eventually transported to the aerial organs of the plant via the vascular system [62, 65]. Unlike the amide assimilation in non-N_2-fixing plants that typically occurs within a single cell, legume nodules partition the pathway by cell type: infected cells perform upstream ammonium assimilation and precursor formation, whereas other cells are enriched for downstream steps that generate the exported N form; together they complete nitrogen synthesis and export (ureides in *G. max*, amides in *M. truncatula*). Studies also showed that uninfected cells in *G. max* nodules originate from peripheral cortical cells, whereas inner cortical cells enriched in key nodulation genes originate from meristematic pericycle-related procambium cells. Notably, the *GmCRE1* gene, which is specifically expressed in inner cortical and vascular cells, exhibits dual regulatory functions; it regulates both the nodule number and efficiency of the subsequent symbiotic nitrogen fixation process [65]. There are significant differences in the nodulation types between *G. max* and *M. truncatula*, primarily determined by the specific cortical cell layer that gives rise to the nodule primordium and by the presence or absence of a persistent meristem. Nevertheless, both species display certain similarities: each nodule type features peripheral vascular bundles and a central zone primarily composed of infected cells, where nitrogen fixation occurs, interspersed with a smaller proportion of uninfected cells [66]. Cell populations in the nitrogen-fixing core region of *G. max* nodules can be categorized into infected non-nitrogen-fixing and infected nitrogen-fixing cell clusters. These cell clusters exhibit functional parallels to cells in zone II and III of *M. truncatula* nodules, respectively; however, this similarity reflects a functional analogy rather than a strict one-to-one correspondence in zonation. Moreover, determinate and indeterminate nodules differ not only in overall morphology (spherical versus cylindrical) but also

in cellular developmental organization. Specifically, in indeterminate nodules, infection, nitrogen fixation, and senescence of the nitrogen-fixing zone are spatially segregated; in contrast, these processes occur concurrently and without spatial compartmentalization in determinate nodules [66]. An snRNA-seq analysis revealed the molecular heterogeneity of cells in the infection zone of *G. max* nodules. Non-nitrogen-fixing cells are primarily regulated by the core symbiotic transcription factor network comprising *NIN*/*NF-YA*/*KNAT/BLH*, whereas infected nitrogen-fixing cells are significantly enriched with genes associated with nodule autoregulation and environmental stress responses. Membrane systems (including the plasma and symbiosome membranes) also play critical roles in nitrogen fixation. The plasma membrane-associated protein GmFWL3 regulates rhizobial infection. This protein is localized not only in the plasma membrane, but also in that of the symbiosome, suggesting that dynamic remodeling of host membrane systems exerts important regulatory effects on symbiotic nitrogen fixation [67].

Cross-species comparative studies have uncovered differences in the expression patterns of key orthologous genes. For example, the *NIN* gene shows distinct interspecies variation in its timing and expression intensity despite being detected in infected cells across *G. max*, *L. japonicus*, and *M. truncatula*. Nevertheless, as a core regulatory factor for symbiotic nitrogen fixation, *NIN* is universally involved in the entire regulatory process from early immune recognition and nodule organogenesis to the establishment of nitrogen fixation functionality [59–62, 64, 67]. This interspecies variation in expression patterns reflects, to some extent, the evolutionary adaptability of symbiotic nitrogen fixation strategies across different species. Overall, current single-cell transcriptome studies have provided an unprecedented resolution for understanding the heterogeneity of nodule cells and uncovered multiple new genes and cell types. However, discrepancies among different studies indicate that more rigorous experimental designs and integrated data analyses are required to better elucidate both universal and species-specific mechanisms underlying nodule development and symbiotic nitrogen fixation.

Mapping spatial and single-cell transcriptional landscapes of nitrogen fixation

Single-cell transcriptome technology has precisely revealed cellular heterogeneity and dynamic changes in gene expression during nodule development. However, disruption of the tissue structure during the experimental process causes the resulting single-cell data to lack clear spatial position information, thereby failing to accurately reflect the spatial pattern of gene expression within the tissues. However, spatial transcriptomics effectively compensates for this limitation by allowing in situ capture and sequencing of tissue sections and visually presenting the distribution patterns of gene expression at the tissue spatial level [68]. This makes spatial transcriptomics particularly suitable for analyzing tissue structures with distinct functional zonation, such as nodules.

For the first time, spatial transcriptomics has been employed in *L. japonicus*-related studies to systematically analyze the dynamic developmental process of the root nodule infection zone from pre-infection through functional maturation. Such studies revealed that *LjNLP3* plays a critical regulatory role in root nodule developmental stage transitions and that its overexpression significantly promotes early nodule maturation. Researchers have demonstrated that the differentiation of peripheral tissues and the infection zone surrounding the two key regions in the root nodule occurs concurrently

in determinate nodules, and this coordinated development is closely associated with the symbiotic nitrogen fixation function of the root nodule. A core gene set involved in this process has been identified, including *SCR* and *PLT*, which are crucial for the regulation of root nodule meristems. Identified spatial co-expression modules indicate that *LjsymLHW* functions as a transcription factor that may play a regulatory role in root nodule development [69]. In addition, specialized structures such as Casparian strips play a critical role in the development of root nodules in *L. japonicus*. The spatial distribution and expression patterns of these structures are fundamental for nodule formation. Notably, spatiotemporal analyses have enabled the dissection of the underlying spatial regulatory networks associated with this process [70].

By combining snRNA-seq and spatial transcriptome data (ST-seq), researchers generated a high-resolution cell atlas of *G. max* nodules. This study revealed the functional specialization of uninfected cells in the central infection zone and identified enrichment patterns of nodulation-related genes in transitionally infected cells. Of particular significance is the discovery of a rare cell subtype that is potentially involved in infection thread extension, and rhizobial release. Functional gene studies have demonstrated that knocking out genes specifically expressed in this cell subtype results in an increased nodule number and whitening of the infection zone, highlighting their essential role in regulating symbiotic interactions [71]. At a broader level, the integration of snRNA-seq and ST-seq has yielded an organ-development atlas of *G. max* across its entire life cycle. In total, 12 spatial and 26 single-cell clusters were identified within the root nodule tissues. Moreover, the expression of symbiotic nitrogen fixation genes such as *ENOD40* was detected in specific cell clusters. Additionally, the knockout of *GmPMTs*, which are specifically expressed in root nodules, significantly decreased the number of root nodules. Known symbiotic nitrogen fixation genes were found to be active in five distinct cell clusters, while two key cluster-specific genes, *GmHB1a* and *GmHB1b*, were identified. GmHB1a is predominantly expressed in cells at the vascular bundle junction of the root nodule, whereas GmHB21 is primarily localized in vascular bundle cells. GmHB1a and GmHB1b regulate the likelihood of infectious events during nodule development [72]. Integrating of scATAC-seq, snRNA-seq, and ST-seq datasets from ten *G. max* tissues revealed 303,199 accessible chromatin regions (ACRs) and 103 distinct cell types [73]. Among these ACRs, 71.9% exhibited activating features, 24.1% repressive characteristics, and 3.9% both positive and negative correlations. Moreover, approximately 40.23% (122,558) were classified as cell type-specific ACRs (ctACRs). Enrichment analysis of transcription factor motifs in ctACRs relative to those in non-ctACRs across different cell types and tissues revealed both known and potentially novel regulatory elements. Researchers have observed that 73 enriched transcription factor motifs are specifically associated with infected cells, including the well-characterized nodule symbiosis regulatory motif *NLP7*, which exhibits non-cell-autonomous activity, and that the *NIN*-centered regulatory network is highly conserved among leguminous plants. The most enriched transcription factor motifs in the infected cell-specific ACRs were AHL13 and ANTHOCYANINLESS 2, which have been implicated in jasmonic acid biosynthesis and signaling and in anthocyanin accumulation and primary root tissue development, respectively [73].

Further integration of epigenomic datasets, including ATAC-seq, histone modifications, and chromatin conformation capture (Hi-C), is anticipated to dissect the epigenetic mechanisms of gene regulatory networks underlying symbiotic nitrogen fixation [74–78]. The application of Hi-C technology in peanut reveals that, relative to roots, approximately 2.0% of chromosomal regions switch from the inactive B compartment (densely packed heterochromatin with low gene density and transcriptional inactivity) to the active A compartment (decondensed euchromatin enriched in genes and marked by active transcription) during nodule development. This shift coincides with significant remodeling of topologically associating domains and increased active cis-regulatory interactions. Together, these features indicate a genome-wide reorganization of three-dimensional chromatin architecture during nodule development [79]. The use of scATAC-seq technology enables precise identification of chromatin accessibility regions specific to distinct cell types during nodulation development. Concurrently, analyses of histone modifications—such as H3K27ac and H3K4me1—can further delineate regulatory elements, including enhancers, associated with these cell types. Integrating Hi-C data allows for the mapping of chromatin loops that interact with nitrogen fixation genes, thereby facilitating the reconstruction of gene regulatory networks underlying root nodule development. Collectively, these epigenetic datasets provide a comprehensive framework for elucidating how transcriptional regulation, epigenetic modifications, and three-dimensional chromatin architecture coordinately govern nitrogen-fixation efficiency at single-cell resolution. Importantly, most studies have focused on a single species, with few incorporating cross-species or cross-nodule-type comparison. Therefore, cross-species comparative studies of spatial transcriptomics in leguminous plants are expected to uncover spatial conservation and divergence in gene expression among different types of root nodules (determinate vs. indeterminate) and diverse symbiotic patterns. These data can provide valuable support for understanding the evolutionary diversity of symbiotic nitrogen fixation based on cellular expression differences. They also provide critical theoretical foundations and technical support for the molecular breeding of high-efficiency nitrogen-fixing crops. Nevertheless, key challenges remain, particularly in overcoming barriers related to host-microbe recognition and oxygen-sensitive regulation mechanisms [80].

Cross-species single-cell insights into nodule type divergence

To elucidate the mechanisms underlying nodule formation and nitrogen fixation, and to identify the minimal set of gene clusters required for these processes, researchers have undertaken a variety of investigative approaches. Based on comparative genomics, two primary evolutionary hypotheses have been proposed concerning the origins of nodulation and nitrogen fixation. Recent phylogenetic analyses suggest that the capacity for symbiotic nitrogen fixation has been independently lost multiple times throughout plant evolution [81]. However, this does not explain how nodules were first developed. Further research has provided relevant insights on the origin of this process, for example, the evolution of *NIN* was used as a case study to elucidate how the transition from *NLP1* and *NLP2* to *NIN* coincides with the emergence of the nitrogen-fixing phenotype [82]. Using corHMM method to estimate evolutionary rates of root nodule symbiosis (RNS) across species, another study proposed a two-step evolutionary process: an ancestral

precursor state first transitioned to a more labile state from which RNS was rapidly gained at multiple points in the NFC. The study provides new evidence for the multiple independent gains and losses of RNS. Lines of the primary genetic and evolutionary evidence supporting multiple origins and multiple losses includes: (1) different species employ distinct genes or pathways; (2) pseudogenes are absent in non-nodulating species, or their distribution is unrelated to the trait; (3) the evolutionary histories of trait-associated genes conflict with the species phylogeny; and (4) a lack of gene collinearity is observed. In contrast, a single origin with multiple losses hypothesis is less consistent with these observations. These data suggest that a broader phylogenetic and genetic framework is necessary for effective genome-phenome mapping [83]. Noteworthy, the non-leguminous plant *Parasponia* independently evolved the ability for symbiotic nitrogen fixation with rhizobia via convergent evolution [84]. Additionally, comparative analyses of core transcription factors such as NIN and NF-YA across species revealed conserved and species-specific regulatory modules. In particular, the loss or silencing of the key initiating transcription factor NIN can explain the evolutionary loss of the nodule phenotype in certain legumes [85]. Comparative transcriptomics further showed that the ancestral RNS transcriptomic signature closely resembles that of extant species, suggesting that it likely evolved from a non-RNS-forming state. A regulatory connection between the common symbiosis pathway and the key transcription factor NIN could have played a crucial role in facilitating this evolutionary transition [86]. These observations indicate that gene presence or absence alone may not capture the complexity of symbiotic nitrogen fixation. Therefore, from a data-analytic perspective, researchers are increasingly investigating the formation and regulation of root nodule symbiosis across multiple dimensions.

Given the cellular heterogeneity within nodule tissues, we propose an analytical framework (perspective)—single-cell comparative transcriptome analysis—that offers high precision and can elucidate the core regulatory network of the legume-rhizobia symbiotic system at the cellular level (Fig. 2). Moreover, comparative single-cell transcriptome analyses primarily address three key scientific questions: (i) formation mechanism differences between determinate and indeterminate nodules and other symbiotic situations, (ii) regulatory pathway similarities and dissimilarities between crack entry and root hair infection, and (iii) the molecular basis underlying variations in nitrogen fixation efficiency across species [87]. By acquiring and comparing single-cell transcriptomic data from root nodules in the leguminous plants, such as *G. max*, *P. sativum*, *M. truncatula*, *L. japonicus*, and *A. hypogaea*, and the non-leguminous plant *P. andersonii*, with corresponding root data from non-nodulating species, including *A. thaliana*, *Fragaria ananassa*, and *Citrullus lanatus* (Fig. 2A), we can perform cross-species single-cell comparative analysis. The initial step is to identify orthologous genes across species and to characterize gene diversity (Fig. 2B). Cluster annotation and expression quantification of single-cell data are then conducted across five representative developmental stages within a single species (Fig. 2C). Cross-species data integration is subsequently performed using CCA/scANVI, SAMap, SATURN, and other relevant methods, after which tools such as XSpeciesSpanner are applied to achieve robust and reliable cross-species cell type annotation (Fig. 2D) [88–90]. Finally, based on the orthologous-gene set, gene expression patterns and regulatory networks are analyzed systematically (Fig. 2E). This

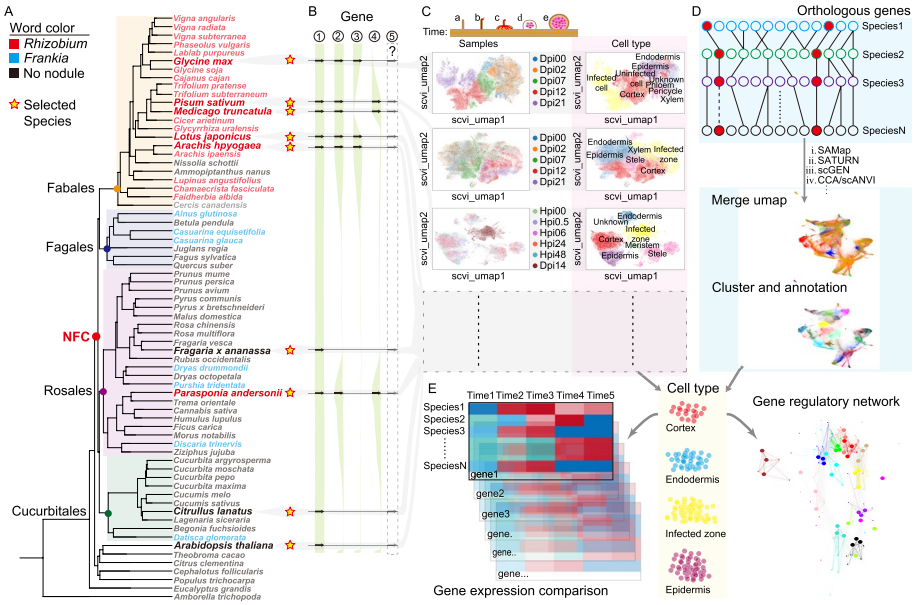

Fig. 2 Comparative single-cell transcriptomic analysis in nitrogen-fixing plants. **A** Phylogenetic tree of species sampled within and outside the nitrogen-fixing clade (NFC), with the four angiosperm orders containing nodulating lineages highlighted by background shading: Fabales (light beige), Fagales (light blue), Rosales (light pink), and Cucurbitales (light green). Species-name font colors indicate symbiotic category: red, Rhizobial nodulators; blue, *Frankia*-associated nodulators; black, non-nodulating taxa. Stars denote species selected for cross-species single-cell comparative analyses. **B** The gene evolutionary model of nitrogen-fixing plants: ① Genes common to all examined plants (including nodulating nitrogen-fixing plants, non-nitrogen-fixing plants in NFC and non-nitrogen-fixing plants as outgroup); ② Genes only existing in nodulating nitrogen-fixing plants; ③ Genes exclusively found in determinate nodule; ④ Genes exclusively found in indeterminate nodule; ⑤ Other cases (species-/lineage-specific). **C** Single-cell transcriptome analyses across five representative developmental stages in different species, including clustering and cell-type annotation. Comparative stages are defined as follows: (a) Uninoculated; (b) Root-hair curling; (c) Nodule primordium formation; (d) Young nodule; (e) Nodule maturation and nitrogen fixation initiation. Note: The labels shown on the UMAPs indicate dataset-specific sampling time points. Hpi00 and Dpi00 (uninoculated controls) correspond to stage (a); Hpi0.5, Hpi06, Hpi24, Hpi48, and Dpi02 correspond to stage (b); Dpi07 to stage (c); Dpi12 and Dpi14 to stage (d); and Dpi21 to stage (e). **D** Integration of single-cell transcriptomic data across different species using multiple software tools based on orthologous genes. Several algorithms were listed as SAMap, SATURN, ScGEN, and CCA/scANVI. **E** Gene expression profiles and regulatory networks reconstructed from single-cell analyses of nodules across different plant species

research framework can systematically dissect cellular-level differences underlying nodule initiation, development, and nitrogen fixation differentiation. Integration with comparative genomic analyses further enables evaluation of phenotypic evolutionary diversity at single-cell resolution. This research strategy provides a comprehensive overview of cell-type-specific gene expression patterns during nodulation and offer valuable insights into the identification and selection of key regulatory genes. As such, they have the potential to address the long-standing challenge of establishing functional nodulation and biological nitrogen fixation in grasses.

Concluding remarks and future perspectives

Single-cell and spatial transcriptomics are reshaping research on symbiotic nitrogen fixation, delivering unprecedented resolution on nodule cell heterogeneity, cellular differentiation, and spatial gene-expression patterns. With continuing technological

advancements and improvements in integrative multi-omics methods, these approaches are poised to transform our understanding of the interactions between legume-rhizobia. Looking ahead, several directions deserve particular attention. First, using single-cell and spatial atlases to map in vivo legume-rhizobia interactions, such as ligand–receptor pairs and metabolite exchange. Second, deploying single-cell multi-omics to reconstruct cell-type-specific regulatory networks by linking enhancers, transcription factors and three-dimensional chromatin contacts. Third, leveraging single-cell and spatial datasets to probe the evolutionary origins of symbiosis. Taken together, the integration of spatial and single-cell transcriptomics with epigenomics, and synthetic biology can drive a pivotal shift from foundational discovery to the molecular breeding of high-efficiency, nitrogen-fixing crops, advancing sustainable agricultural development.

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Authors' contributions
H.W., J.Z., and W.Y. conceived this project. H.W. supervised this project. Y.S., and H.L. collected the materials and wrote the draft. H.W., and J.Z. revised this manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

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References

1. Xu P, Wang E. Diversity and regulation of symbiotic nitrogen fixation in plants. Curr Biol. 2023;33:R543–59.
2. Oldroyd GED, Downie JA. Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol. 2008;59:519–46.
3. Hiltner L. Über die Bedeutung der Wurzelknöllchen von Alnus glutinosa für die Stickstoffernährung dieser Pflanze. Landw Versuchsstat. 1895;46:153–61.
4. Hellriegel H, Wilfarth H. Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. Berlin: Buchdruckerei der Post Kayssler; 1888.
5. Soltis DE, Soltis PS, Morgan DR, Swensen SM. Martin PGJPotNAoS: chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. PNAS. 1995;92:2647–51.
6. Kistner C, Parniske M. Evolution of signal transduction in intracellular symbiosis. Trends Plant Sci. 2002;7:511–8.
7. Doyle JJ. Phylogenetic perspectives on the origins of nodulation. Mol Plant-Microbe Interact. 2011;24:1289–95.
8. Sprent JI, Ardley J, James EKJNP. Biogeography of nodulated legumes and their nitrogen-fixing symbionts. New Phytol. 2017;215:40–56.
9. Pawlowski K, Demchenko KNJP. The diversity of actinorhizal symbiosis. Protoplasma. 2012;249:967–79.
10. Roy S, Liu W, Nandety RS, Crook A, Mysore KS, Pislariu CI, et al. Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. Plant Cell. 2020;32:15–41.
11. Murray JD. Invasion by invitation: rhizobial infection in legumes. Mol Plant-Microbe Interact. 2011;24:631–9.
12. Gage DJ. Nodule development in legumes. In: Emerich DW, Krishnan HB, editors. Nitrogenfi xation in crop production. Madison, WI: American Society of Agronomy; 2009. p. 1–24.

13. de Faria SM, Ringelberg JJ, Gross E, Koenen EJM, Cardoso D, Ametsitsi GKD, et al. The innovation of the symbiosome has enhanced the evolutionary stability of nitrogen fixation in legumes. New Phytol. 2022;235:2365–77.
14. Zipfel C. Plant pattern-recognition receptors. Trends Immunol. 2014;35:345–51.
15. Lopez-Gomez M, Sandal N, Stougaard J, Boller TJJoEB. Interplay of flg22-induced defence responses and nodulation in *Lotus japonicus*. J Exp Bot. 2011;63:393–401.
16. Jamet A, Mandon K, Puppo A, Hérouart D. H_2O_2 is required for optimal establishment of the *Medicago sativa/Sinorhizobium meliloti* symbiosis. J Bacteriol. 2007;189:8741–5.
17. Andrio E, Marino D, Marmeys A, Dunoyer de M, Phytologist SJN. Hydrogen peroxide-regulated genes in the *Medicago truncatula–Sinorhizobium meliloti* symbiosis. New Phytol. 2013;198:190–202.
18. Wasson AP, Pellerone FI, Mathesius U. Silencing the flavonoid pathway in *Medicago truncatula* inhibits root nodule formation and prevents auxin transport regulation by rhizobia. Plant Cell. 2006;18:1617–29.
19. Schauser L, Roussis A, Stiller J, Stougaard J. A plant regulator controlling development of symbiotic root nodules. Nature. 1999;402:191–5.
20. Cao Y, Halane MK, Gassmann W, Stacey G. The role of plant innate immunity in the legume-rhizobium symbiosis. Annu Rev Plant Biol. 2017;68:535–61.
21. Okamoto S, Ohnishi E, Sato S, Takahashi H, Kawaguchi MJP, Physiology C. Nod factor/nitrate-induced CLE genes that drive HAR1-mediated systemic regulation of nodulation. Plant Cell Physiol. 2009;50:67–77.
22. Mortier V, Den Herder G, Whitford R, Van de Velde W, Rombauts S, D'haeseleer K, Holsters M, Goormachtig S. CLE peptides control *Medicago truncatula* nodulation locally and systemically. Plant Physiol. 2010;153:222–37.
23. Wang D, Jin R, Shi X, Guo H, Tan X, Zhao A, et al. A kinase mediator of rhizobial symbiosis and immunity in Medicago. Nature. 2025;643:768–75.
24. Dong W, Zhu Y, Chang H, Wang C, Yang J, Shi J, et al. An SHR–SCR module specifies legume cortical cell fate to enable nodulation. Nature. 2021;589:586–90.
25. Libault M, Farmer A, Brechenmacher L, Drnevich J, Langley RJ, Bilgin DD, et al. Complete transcriptome of the soybean root hair cell, a single-cell model, and its alteration in response to *Bradyrhizobium japonicum* infection plant physiol. Plant Physiol. 2010;152:541–52.
26. Breakspear A, Liu C, Roy S, Stacey N, Rogers C, Trick M, et al. 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. 2014;26:4680–701.
27. Roux B, Rodde N, Jardinaud MF, Timmers T, Sauviac L, Cottret L, et al. An integrated analysis of plant and bacterial gene expression in symbiotic root nodules using laser-capture microdissection coupled to RNA sequencing. Plant J. 2014;77:817–37.
28. Schiessl K, Lilley JLS, Lee T, Tamvakis I, Kohlen W, Bailey PC, et al. NODULE INCEPTION recruits the lateral root developmental program for symbiotic nodule organogenesis in *Medicago truncatula*. Curr Biol. 2019;29:3657-3668.e3655.
29. Shaw R, Tian X, Xu J. Single-cell transcriptome analysis in plants: advances and challenges. Mol Plant. 2021;14:115–26.
30. Yin R, Xia K, Xu X. Spatial transcriptomics drives a new era in plant research. Plant J. 2023;116:1571–81.
31. Ma S, Ji Z, Zhang B, Geng L, Cai Y, Nie C, et al. Spatial transcriptomic landscape unveils immunoglobin-associated senescence as a hallmark of aging. Cell. 2024;187:7025-7044.e7034.
32. Tadross JA, Steuernagel L, Dowsett GKC, Kentistou KA, Lundh S, Porniece M, et al. A comprehensive spatio-cellular map of the human hypothalamus. Nature. 2025;639:708–16.
33. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, et al. Molecular architecture of the mouse nervous system. Cell. 2018;174:999-1014.e1022.
34. Emont MP, Jacobs C, Essene AL, Pant D, Tenen D, Colleluori G, et al. A single-cell atlas of human and mouse white adipose tissue. Nature. 2022;603:926–33.
35. Zhang Z, Schaefer C, Jiang W, Lu Z, Lee J, Sziraki A, et al. A panoramic view of cell population dynamics in mammalian aging. Science. 2025;387:6731.
36. Sun Z, Jiang S, Wang D, Li L, Liu B, Ran Q, et al. Single-cell RNA-seq of *Lotus japonicus* provide insights into identification and function of root cell types of legume. J Integr Plant Biol. 2023;65:1147–52.
37. Wang L, Zhou Y, Li R, Liang J, Tian T, Ji J, et al. Single cell-type transcriptome profiling reveals genes that promote nitrogen fixation in the infected and uninfected cells of legume nodules. Plant Biotechnol J. 2022;20:616–8.
38. Denyer T, Ma X, Klesen S, Scacchi E, Nieselt K, Timmermans MCP. Spatiotemporal developmental trajectories in the *Arabidopsis* root revealed using high-throughput single-cell RNA sequencing. Dev Cell. 2019;48:840-852.e845.
39. Liew LC, You Y, Auroux L, Oliva M, Peirats-Llobet M, Ng S, et al. Establishment of single-cell transcriptional states during seed germination. Nat Plants. 2024;10:1418–34.
40. Wang L, Wan MC, Liao RY, Xu J, Xu ZG, Xue HC, et al. The maturation and aging trajectory of *Marchantia polymorpha* at single-cell resolution. Dev Cell. 2023;58:1429-1444.e1426.
41. Tung CC, Kuo SC, Yang CL, Yu JH, Huang CE, Liou PC, et al. Single-cell transcriptomics unveils xylem cell development and evolution. Genome Biol. 2023;24:3.
42. Song J, Fan B, Shao X, Zang Y, Wang D, Min Y. Single-cell transcriptome sequencing atlas of cassava tuberous root. Front Plant Sci. 2023;13:1053669.
43. Guillotin B, Rahni R, Passalacqua M, Mohammed MA, Xu X, Raju SK, et al. A pan-grass transcriptome reveals patterns of cellular divergence in crops. Nature. 2023;617:785–91.
44. Zong J, Wang L, Zhu L, Bian L, Zhang B, Chen X, et al. A rice single cell transcriptomic atlas defines the developmental trajectories of rice floret and inflorescence meristems. New Phytol. 2022;234:494–512.
45. Zhou P, Chen H, Dang J, Shi Z, Shao Y, Liu C, et al. Single-cell transcriptome of *Nepeta tenuifolia* leaves reveal differentiation trajectories in glandular trichomes. Front Plant Sci. 2022;13:988594.
46. Xia K, Sun H-X, Li J, Li J, Zhao Y, Chen L, et al. The single-cell stereo-seq reveals region-specific cell subtypes and transcriptome profiling in *Arabidopsis* leaves. Dev Cell. 2022;57:1299-1310.e1294.
47. Wang Q, Wu Y, Peng A, Cui J, Zhao M, Pan Y, et al. Single-cell transcriptome atlas reveals developmental trajectories and a novel metabolic pathway of catechin esters in tea leaves. Plant Biotechnol J. 2022;20:2089–106.

48. Tao S, Liu P, Shi Y, Feng Y, Gao J, Chen L, et al. Single-cell transcriptome and network analyses unveil key transcription factors regulating mesophyll cell development in maize. Genes. 2022;13:374.
49. Sun X, Feng D, Liu M, Qin R, Li Y, Lu Y, et al. Single-cell transcriptome reveals dominant subgenome expression and transcriptional response to heat stress in Chinese cabbage. Genome Biol. 2022;23:262.
50. Guo X, Wang Y, Zhao C, Tan C, Yan W, Xiang S, et al. An *Arabidopsis* single-nucleus atlas decodes leaf senescence and nutrient allocation. Cell. 2025;188(11):2856-2871.e16.
51. Swift J, Luginbuehl LH, Hua L, Schreier TB, Donald RM, Stanley S, et al. Exaptation of ancestral cell-identity networks enables C4 photosynthesis. Nature. 2024;636:143–50.
52. Yao J, Chu Q, Guo X, Shao W, Shang N, Luo K, et al. Spatiotemporal transcriptomic landscape of rice embryonic cells during seed germination. Dev Cell. 2024;59:2320-2332.e2325.
53. Wang Y, Luo Y, Guo X, Li Y, Yan J, Shao W, et al. A spatial transcriptome map of the developing maize ear. Nat Plants. 2024;10:815–27.
54. Farmer A, Thibivilliers S, Ryu KH, Schiefelbein J, Libault M. Single-nucleus RNA and ATAC sequencing reveals the impact of chromatin accessibility on gene expression in *Arabidopsis* roots at the single-cell level. Mol Plant. 2021;14:372–83.
55. Marand AP, Chen Z, Gallavotti A, Schmitz RJ. A cis-regulatory atlas in maize at single-cell resolution. Cell. 2021;184:3041-3055.e3021.
56. Soyano T, Shimoda Y, Kawaguchi M, Hayashi M. A shared gene drives lateral root development and root nodule symbiosis pathways in *Lotus*. Science. 2019;366:1021–3.
57. Serrano-Ron L, Perez-Garcia P, Sanchez-Corrionero A, Gude I, Cabrera J, Ip P-L, et al. Reconstruction of lateral root formation through single-cell RNA sequencing reveals order of tissue initiation. Mol Plant. 2021;14:1362–78.
58. Gala HP, Lanctot A, Jean-Baptiste K, Guiziou S, Chu JC, Zemke JE, et al. A single-cell view of the transcriptome during lateral root initiation in *Arabidopsis thaliana*. Plant Cell. 2021;33:2197–220.
59. Cervantes-Pérez SA, Thibivilliers S, Laffont C, Farmer AD, Frugier F, Libault M. Cell-specific pathways recruited for symbiotic nodulation in the *Medicago truncatula* legume. Mol Plant. 2022;15:1868–88.
60. Liu Z, Yang J, Long Y, Zhang C, Wang D, Zhang X, et al. Single-nucleus transcriptomes reveal spatiotemporal symbiotic perception and early response in *Medicago*. Nat Plants. 2023;9:1734–48.
61. Pereira WJ, Boyd J, Conde D, Triozzi PM, Balmant KM, Dervinis C, et al. The single-cell transcriptome program of nodule development cellular lineages in *Medicago truncatula*. Cell Rep. 2024;43:113747.
62. Ye Q, Zhu F, Sun F, Wang T-C, Wu J, Liu P, et al. Differentiation trajectories and biofunctions of symbiotic and unsymbiotic fate cells in root nodules of *Medicago truncatula*. Mol Plant. 2022;15:1852–67.
63. Zhang X, Wu J, Kong Z. Cellular basis of legume–rhizobium symbiosis. Plant Commun. 2024;5:101045.
64. Frank M, Fechete LI, Tedeschi F, Nadzieja M, Nørgaard MMM, Montiel J, et al. Single-cell analysis identifies genes facilitating rhizobium infection in *Lotus japonicus*. Nat Commun. 2023;14:7171.
65. Sun B, Wang Y, Yang Q, Gao H, Niu H, Li Y, et al. A high-resolution transcriptomic atlas depicting nitrogen fixation and nodule development in soybean. J Integr Plant Biol. 2023;65:1536–52.
66. Hirsch AM. Developmental biology of legume nodulation. New Phytol. 1992;122:211–37.
67. Cervantes-Pérez SA, Zogli P, Amini S, Thibivilliers S, Tennant S, Hossain MS, et al. Single-cell transcriptome atlases of soybean root and mature nodule reveal new regulatory programs that control the nodulation process. Plant Commun. 2024;5:100984.
68. You Y, Fu Y, Li L, Zhang Z, Jia S, Lu S, et al. Systematic comparison of sequencing-based spatial transcriptomic methods. Nat Methods. 2024;21:1743–54.
69. Ye K, Bu F, Zhong L, Dong Z, Ma Z, Tang Z, et al. Mapping the molecular landscape of *Lotus japonicus* nodule organogenesis through spatiotemporal transcriptomics. Nat Commun. 2024;15:6387.
70. Shen D, Micic N, Venado RE, Bjarnholt N, Crocoll C, Persson DP, et al. Apoplastic barriers are essential for nodule formation and nitrogen fixation in *Lotus japonicus*. Science. 2025;387:1281–6.
71. Liu Z, Kong X, Long Y, Liu S, Zhang H, Jia J, et al. Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation. Nat Plants. 2023;9:515–24.
72. Fan J, Shen Y, Chen C, Chen X, Yang X, Liu H, et al. A large-scale integrated transcriptomic atlas for soybean organ development. Mol Plant. 2025;18:669–89.
73. Zhang X, Luo Z, Marand AP, Yan H, Jang H, Bang S, et al. A spatially resolved multi-omic single-cell atlas of soybean development. Cell. 2025;188:550-567.e519.
74. McGarvey AC, Kopp W, Vucicevic D, Mattonet K, Kempfer R, Hirsekorn A, et al. Single-cell-resolved dynamics of chromatin architecture delineate cell and regulatory states in zebrafish embryos. Cell Genom. 2022;2:100083.
75. Preissl S, Gaulton KJ, Ren B. Characterizing cis-regulatory elements using single-cell epigenomics. Nat Rev Genet. 2023;24:21–43.
76. Zu S, Li YE, Wang K, Armand EJ, Mamde S, Amaral ML, et al. Single-cell analysis of chromatin accessibility in the adult mouse brain. Nature. 2023;624:378–89.
77. Bartosovic M, Kabbe M, Castelo-Branco G. Single-cell CUT&tag profiles histone modifications and transcription factors in complex tissues. Nat Biotechnol. 2021;39:825–35.
78. Li C, Wood JC, Vu AH, Hamilton JP, Rodriguez Lopez CE, Payne RME, et al. Single-cell multi-omics in the medicinal plant *Catharanthus roseus*. Nat Chem Biol. 2023;19:1031–41.
79. Wang L, Mai C, He S, Niu B, Jia G, Yang T, et al. Dynamic 3D chromatin organization and epigenetic regulation of gene expression in peanut nodules. J Integr Plant Biol. 2025;67:2624–42.
80. Rogers C, Oldroyd GED. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J Exp Bot. 2014;65:1939–46.
81. Griesmann M, Chang Y, Liu X, Song Y, Haberer G, Crook MB, et al. Phylogenomics reveals multiple losses of nitrogen-fixing root nodule symbiosis. Science. 2018;361:eaat1743.
82. Zhang Y, Fu Y, Xian WF, Li XL, Feng Y, Bu FJ, et al. Comparative phylogenomics and phylotranscriptomics provide insights into the genetic complexity of nitrogen-fixing root-nodule symbiosis. Plant Commun. 2024;5:100671.

83. Kates HR, O'Meara BC, LaFrance R, Stull GW, James EK, Liu S-Y, et al. Shifts in evolutionary lability underlie independent gains and losses of root-nodule symbiosis in a single clade of plants. Nat Commun. 2024;15:4262.
84. van Velzen R, Holmer R, Bu F, Rutten L, van Zeijl A, Liu W, et al. Comparative genomics of the nonlegume Parasponia reveals insights into evolution of nitrogen-fixing rhizobium symbioses. Proc Natl Acad Sci U S A. 2018;115:E4700–9.
85. Yu H, Lu Y, Zhang C, Yang W, Xie H, Liu H, et al. Genomic insights into the absence of root nodule formation and nitrogen fixation in *Zenia insignis*. J Genet Genomics. 2024;52:860–3.
86. Libourel C, Keller J, Brichet L, Cazale AC, Carrere S, Vernie T, et al. Comparative phylotranscriptomics reveals ancestral and derived root nodule symbiosis programmes. Nat Plants. 2023;9:1067–80.
87. Serrano K, Bezrutczyk M, Goudeau D, Dao T, O'Malley R, Malmstrom RR, et al. Spatial co-transcriptomics reveals discrete stages of the arbuscular mycorrhizal symbiosis. Nat Plants. 2024;10:673–88.
88. Tarashansky AJ, Musser JM, Khariton M, Li P, Arendt D, Quake SR, et al. Mapping single-cell atlases throughout Metazoa unravels cell type evolution. Elife. 2021;10:e66747.
89. Rosen Y, Brbic M, Roohani Y, Swanson K, Li Z, Leskovec J. Toward universal cell embeddings: integrating single-cell RNA-seq datasets across species with SATURN. Nat Methods. 2024;21:1492–500.
90. Xue HC, Xu ZG, Liu YJ, Wang L, Ming X, Wu ZY, et al. A unified cell atlas of vascular plants reveals cell-type foundational genes and accelerates gene discovery. Cell. 2025;188:6370-6390.e29.

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