Single-Cell Transcriptomic Analysis of Root Development under Nitrogen Deficiency in *Zea mays* L.: A Critical Review and Synthesis

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ABSTRACT

The problem of nitrogen deficiency remains one of the key limiting factors in the overall productivity of maize (*Zea mays* L.) all around the world, as it is very important to follow how certain root cell types "see" and react to nitrogen deficiency for the sake of improving nitrogen-use efficiency (NUE) in such crops that utilize huge amounts of nitrogen fertilizers. The technique of single-cell RNA sequencing (scRNAseq) is widely used for the purpose of obtaining data concerning the cell type-specific expression of genes which cannot be done with the help of bulk transcriptomic techniques because of their averaging of information received from a mixed root tissue. The review gathered data from the scientific literature concerning single-cell transcriptomic studies of maize and other plants in relation to root development and nitrogen deficiency response. It was actively pointed to in the previous studies that nine to eleven different root cell types have been identified thanks to maize root scRNAseq methods such as the epidermis, root hair, cortex, endodermis, pericycle, and vascular tissue with different types of marker gene expression. It was shown that the information concerning the expression of genes linked to nitrate transception and transport, components epidermal cells. The review also highlights important shortcomings, such as the dominance of many basic scRNA-seq techniques in Arabidopsis as opposed to maize, relatively few studies of maize root scRNA-seq that examine effects specifically related to nitrogen deficiency despite the huge amount of available literature regarding nitrate's impact, and limited success in converting potentially useful candidate genes identified in scRNA-seq studies into breeding targets for the enhancement of nitrogen-use efficiency. It is emphasized, therefore, that single-cell transcriptomics can provide a valid and increasingly practical scientific framework for the investigation of cell type-related reactions to nitrogen in maize roots, and several key points have been identified for application of scRNA-seq data to developing actual breeding targets based on identified genes and other candidate genes.

Keywords: scRNA-seq; Cell-type-specific gene expression; Nitrate signaling; Root system architecture; Functional genomics; Transcriptional regulatory networks; Nitrogen-use efficiency; Developmental trajectory analysis

1. Introduction

The world's cereal crop with the highest production amount is maize (*Zea mays* L.). Today, the crop is increasingly important for global food and animal feed supply chain. More specifically, maize employs excessive amounts of nitrogen in comparison to its peers in agriculture. Although maize receives most of the global nitrogen fertilization, with 30-50% of it being recovered inefficiently, maize production exhibits constant nitrogen losses through leaching and volatilization, which is a cost for farmers, as well as creates nitrogen pollution (Gaudinier *et al*., 2018) [21] (de Bang *et al*., 2021) [23] (Coruzzi and Bush, 2001) [33].

The architecture of a maize root has an important role in determining how the crop gets the nitrogen from soil. The crop's efficiency in nitrogen acquisition from soil depends mainly on root plasticity in pivotal situations. In normal situations, cereals develop certain strategies for the roots of maize, making them active and covering large soil areas efficiently (Yu *et al*., 2016) [20] (Sattelmacher *et al*., 1993) [22] (Cai *et al*., 2012) [26]. In the process of acquiring nitrogen, plants rely on intricate molecular signaling, which makes it possible for root plasticity to happen.

The process of nitrogen uptake and assimilation takes place in maize roots through the action of systems involved in nitrate and ammonium transport, i.e. the NRT1/NPF, NRT2, and AMT gene families. Then, the assimilated nitrogen is further incorporated into organic compounds using the activities of nitrate reductase, nitrite reductase, and GS-GOGAT cycle enzymes. However, important to note is that this series of events does not occur in the same way among different types of roots due to fact that different root tissue types perform different reactions and therefore bring about different outcomes depending on type of the root tissue cell in which this gene expression takes place (Li *et al*., 2022) [1] (Bian *et al*., 2025) [2].

Single-cell transcriptomics (scRNA-seq) overcomes the limitation of bulk tissue homogenates by profiling gene expression in individual cells. This allows for the identification of different cell populations and their respective marker genes, as well as the responses of these populations to experimental conditions. Following a brief cycle of proof-of-concept applications with small numbers of cells, droplet-and microfluidics-based scRNA-seq technologies led to a series of major advances in whole-organ single-cell studies starting in 2019 when more than 100,000 cells were profiled in Arabidopsis root tissue, marking the introduction of fundamental principles for the analysis of scRNA-seq data in plants, including protoplast preparation, cluster-annotations and pseudotime trajectory reconstruction (Denyer *et al*., 2019) [3] (Jean-Baptiste *et al*., 2019) [4] (Ryu *et al*., 2019) [5] (Shulse *et al*., 2019) [6] (Zhang *et al*., 2019) [7].

The application of scRNA-seq in maize and other crops has occurred somewhat later than its implementation in Arabidopsis, with further challenges arising from the difficulties in protoplast preparation of cereal root material compared to that of A. thaliana, and the fact that functional genomics tools are less advanced for maize than for Arabidopsis. However, recent studies employing maize root scRNA-seq have identified nine to eleven major types of root cells and characterized cell-specific responses to nitrate supply, fungal attack and other

Functional genomics methodologies utilizing these single-cell datasets are being utilized more and more in studies aimed at pinpointing potential genes and regulatory centers responsible for nitrogen responsive root development, ultimately contributing directly to the field of molecular breeding for enhanced nitrogen-use efficiency (Li *et al*., 2022) [1] (Gaudinier *et al*., 2018) [21] (Cao *et al*., 2023) [30] (Rich-Griffin *et al*., 2020) [32].

Nonetheless, there are still some notable gaps in research. Studies employing direct single-cell transcriptomic techniques that focus on nitrogen deficiency and are conducted in maize roots are still less common compared to both related research on enzymology and physiology of nitrogen response and various bodies of research on Arabidopsis's nitrate response (Li *et al*., 2022) [1] (Krouk *et al*., 2010) [13] (Vidal *et al*., 2020) [15] (Liu *et al*., 2017) [16] (O'Brien *et al*., 2016) [17] (Alvarez *et al*., 2014) [18] (Vidal *et al*., 2013) [19].

This review tackles the existing challenges in single-cell transcriptomics focused on root growth in response to nitrogen deficit, particularly in *Zea mays* L. Therefore, the review is aimed at achieving the following goals: (i) outlining the main principles and the conceptual framework of single-cell transcriptomic analysis when applied to plant root systems; (ii) synthesizing data on discovered populations of cell types and gene expression unique in response to nitrogen deficit; (iii) synthesizing the available evidence regarding signaling cascades in plants responding to nitrogen deficiency; and (iv) critically evaluating the gaps in knowledge and setting new objectives in the application of plant single-cell functional genomics in order to enhance nitrogen-use efficiency in maize cultivation.

2. Single-Cell Transcriptomic Analysis: Conceptual Framework and Methodological Foundations

Single-cell transcriptomic profiling of plant tissue conceptually proceeds through several linked stages: tissue dissociation into a suspension of individual cells or protoplasts, capture and library preparation for individual cells (most commonly using droplet-based microfluidics platforms), sequencing of the resulting cell-barcoded transcript libraries, and computational analysis encompassing quality filtering, dimensionality reduction, clustering, cell-type annotation using known marker genes, differential expression analysis, and, where developmental relationships are of interest, pseudotime trajectory inference (Ryu *et al*., 2019) [5] (Shulse *et al*., 2019) [6] (Cuperus, 2022) [12]. Plant tissue dissociation carries a distinguishing technical consideration relative to animal scRNA-seq: the plant cell wall must be enzymatically removed to generate protoplasts prior to single-cell capture, a process that itself alters the expression of several hundred genes and must be accounted for in downstream analysis and interpretation (Denyer *et al*., 2019) [3] (Shahan *et al*., 2022) [8].

Dimensionality reduction and clustering techniques, most commonly variants of principal component analysis followed by graph-based clustering and non-linear visualization methods such as UMAP or t-SNE, group cells with similar transcriptional profiles into discrete clusters that are then annotated to specific cell types or tissue identities using previously characterized marker genes (Jean-Baptiste *et al*., 2019) [4] (Zhang *et al*., 2019) [7] (Rich-Griffin *et al*., 2020) [32]. Cluster number and resolution are influenced by the number of cells captured and sequencing depth, meaning that comparisons of cell-type diversity across independent studies must account for these technical parameters rather than assuming differences in cluster number directly reflect differences in underlying biological cell-type diversity (Zhang *et al*., 2019) [7].

Pseudotime and trajectory inference methods, most notably approaches originating from the Monocle framework, order individual cells along an inferred developmental continuum based on transcriptional similarity, allowing reconstruction of differentiation trajectories, such as the progression from meristematic initial cells through elongation to terminally differentiated cortex, epidermis, or vascular cell states, without requiring direct temporal sampling of the same cells over time (Jean-Baptiste *et al*., 2019) [4] (Trapnell *et al*., 2014) [29]. This approach is particularly well suited to root systems, where cells at different developmental stages are spatially arranged along the longitudinal root axis, providing a natural, tractable system for trajectory reconstruction (Denyer *et al*., 2019) [3] (Jean-Baptiste *et al*., 2019) [4].

Foundational whole-organ single-cell atlases of the Arabidopsis root, published independently by several research groups beginning in 2019 and later integrated into comprehensive, multi-study reference atlases, established the core methodological and analytical principles now applied across plant scRNA-seq research generally, including maize (Denyer *et al*., 2019) [3] (Jean-Baptiste *et al*., 2019) [4] (Ryu *et al*., 2019) [5] (Shulse *et al*., 2019) [6] (Zhang *et al*., 2019) [7] (Shahan *et al*., 2022) [8]. Comparable single-cell atlases have since been generated for rice roots, revealing both conserved and divergent aspects of root cell-type organization and differentiation relative to Arabidopsis, and providing an important intermediate reference point for interpreting maize root scRNA-seq findings Related single-cell approaches applied to shoot and stem-cell organizing tissues, including maize shoot apical meristem profiling, have further demonstrated that single-cell resolution can resolve fine-grained stem-cell organization and differentiation states beyond the root system specifically (Satterlee *et al*., 2020) [10]. given the closer evolutionary relationship between maize and rice as monocot cereals (Liu *et al*., 2021) [9].

Table 1: Foundational Single-Cell RNA Sequencing Studies of Plant Root Systems Relevant to Maize Nitrogen-Deficiency Research

Study / Species	Approach	Key Contribution	Representative Sources
Denyer *et al*. (Arabidopsis)	Droplet-based scRNA-seq, whole root	Spatiotemporal developmental trajectories across root cell types	[3]
Jean-Baptiste *et al*. (Arabidopsis)	scRNA-seq, 3,121 root cells, Monocle 3 analysis	Dynamics of gene expression across single root cells	[4]
Ryu *et al*. (Arabidopsis)	scRNA-seq, root tips	Molecular relationships among individual plant cells	[5]
Shulse *et al*. (Arabidopsis)	High-throughput scRNA-seq, multiple cell types	Broad cell-type profiling across plant organs	[6]
Zhang *et al*. (Arabidopsis)	scRNA-seq developmental landscape profiling	Integrated developmental landscape of Arabidopsis root	[7]
Shahan *et al*. (Arabidopsis)	Integrated multi-study atlas, 110,000+ cells	Organ-scale atlas with cell identity mutant analysis	[8]
Liu *et al*. (rice)	scRNA-seq + chromatin accessibility, root	Differentiation trajectories in a cereal root system	[9]
Li *et al*. (maize)	scRNA-seq, >7,000 root tip cells, +/- nitrate	11 cell types; 85 cell type-specific nitrate-response genes	[1]
Bian *et al*. (maize)	scRNA-seq, root tips	9 cell types/10 clusters; STP4 cortex hub gene identified	[2]

Table 2: Conceptual Experimental Design Elements Relevant to Single-Cell Nitrogen-Deficiency Studies in Maize Roots, as Synthesized from the Reviewed Literature

Design Element	Typical Reported / Conceptual Approach	Representative Sources
Nitrogen treatments	Nitrate-supplied vs. nitrate-free (or low-N) growth media/soil	[1, 26]
Sampling tissue	Root tips (meristematic and elongation zones)	[1, 2]
Cell dissociation	Enzymatic protoplasting prior to single-cell capture	[3, 8]
Profiling platform	Droplet-based microfluidics single-cell capture and sequencing	[5, 6]
Comparative analysis	Cross-species comparison (e.g., maize vs. rice orthologs)	[1, 9]
Developmental analysis	Pseudotime trajectory reconstruction	[1, 4, 29]

3. Root Cell Population Identification and Cell-Type-Specific Nitrogen Responses in Maize

Direct application of scRNA-seq to maize root tissue has produced an increasingly detailed picture of root cellular organization. Li *et al*. [1] profiled transcriptomes from more than 7,000 cells derived from maize seedling root tips grown with or without nitrate supply, identifying 11 major cell types or tissues and, critically, 85 cell type-specific nitrate-response genes, including several previously characterized nitrate metabolic genes, demonstrating directly that nitrate availability reshapes gene expression in a strongly cell-type-dependent manner rather than uniformly across root tissue. This study further reported that pseudotime analysis placed meristematic zone cells at the origin of a continuous developmental trajectory, with root hair cells arising through differentiation of a specific subset of epidermal cells, and that interspecies comparison with rice root cells identified dozens of conserved orthologous genes in root hair, endodermis, and phloem cell populations, indicating meaningful conservation of cell-type-specific gene expression programs across cereal species (Li *et al*., 2022) [1].

Bian *et al*. [2] extended this characterization using scRNA-seq of maize root tips, identifying nine cell types and ten transcriptionally distinct clusters based on marker and cluster-specific gene expression, with cyclin gene profiling indicating M-phase enrichment concentrated in the meristematic zone, consistent with active cell division at that location. This study further reported that hormone-related gene expression patterns in maize root cell types diverged notably from patterns previously described in Arabidopsis and rice, suggesting species-specific aspects of hormonal regulation of root cell identity and function, and identified the sugar transport protein-encoding gene STP4 as a co-expression network hub gene specifically within the mature cortex, with functional inference suggesting a role in supporting early seedling growth through glucose transport supporting glycolysis and the tricarboxylic acid cycle (Bian *et al*., 2025) [2].

Beyond nitrogen-specific responses, maize root scRNA-seq has also been applied to characterize cell type-specific transcriptional regulatory networks governing fungal invasion, demonstrating the broader utility of the approach for resolving spatially and cellularly restricted responses to diverse biotic and abiotic factors beyond nitrogen status alone (Cao *et al*., 2023) [30]. Collectively, these maize-specific studies establish that root cell-type diversity identified by scRNA-seq (9-11 major populations depending on study parameters) is broadly consistent with the anatomically defined cell types long recognized in root developmental biology, epidermis, cortex, endodermis, pericycle, and vascular tissue, while adding substantial new resolution regarding the specific genes and regulatory programs that define and distinguish these populations transcriptionally (Li *et al*., 2022) [1] (Bian *et al*., 2025) [2]. A structured summary of root cell populations and their characteristic markers and functions, synthesized from the reviewed literature, is presented in Table 3.

Table 3: Root Cell Populations Identified by Single-Cell Transcriptomic Analysis in Maize and Comparator Species

Cell Population	Representative Marker/Hub Genes	Cellular Function	Representative Sources
Epidermis / root hair	Root hair differentiation markers	Nutrient/water absorption interface; nitrate/ammonium uptake	[1, 2]
Cortex (outer/inner)	STP4 (sugar transport protein, cortex hub gene)	Radial transport, storage, glycolysis/TCA-linked metabolism	[2]
Endodermis	Casparian strip-associated genes	Selective radial transport barrier, nutrient filtering	[1, 3]
Pericycle	Lateral root initiation-associated genes	Lateral root primordium initiation site	[1, 13]
Vascular tissue (xylem/phloem)	Phloem/xylem differentiation markers	Long-distance nitrogen and photosynthate transport	[1, 9]
Meristem / root cap	Cyclin genes (M-phase markers)	Active cell division, root growth, cell fate origin	[1, 2]

4. Nitrogen-Responsive Signaling Pathways, Transporters, and Transcriptional Regulators

Nitrate sensing in plant roots begins with the dual-affinity nitrate transceptor NRT1.1 (also known as NPF6.3, or CHL1), which functions both as a nitrate transporter and as a primary nitrate sensor capable of triggering downstream signaling independent of its transport activity, including nitrate-regulated auxin transport that shapes lateral root elongation into nitrate-rich soil patches (Krouk *et al*., 2010) [13] (Gojon *et al*., 2011) [14]. Downstream of nitrate perception, a calcium-dependent protein kinase (CPK)-NIN-like protein (NLP) signaling module has been identified as a central hub linking nitrate sensing to broad transcriptional reprogramming of nitrogen-responsive genes, representing one of the most significant recent advances in understanding the systems-level architecture of plant nitrogen signaling (Liu *et al*., 2017) [16].

High-affinity nitrate uptake under nitrogen-limited conditions is mediated primarily by NRT2 family transporters, which additionally play a signaling role in lateral root initiation that is at least partially independent of their nitrate transport function (Krouk *et al*., 2010) [13] (Gojon *et al*., 2011) [14] (Yu *et al*., 2016) [20]. Ammonium uptake proceeds through AMT1 family transporters, with local ammonium supply shown to stimulate lateral root emergence through an AMT1;3-dependent mechanism in Arabidopsis, a pathway with clear conceptual relevance to maize, though direct maize-specific characterization of this precise mechanism remains more limited (Yu *et al*., 2016) [20].

Transcription factor families implicated in nitrogen-responsive root development and cell-type-specific gene regulation include LBD (Lateral Organ Boundaries Domain) family members, which have been proposed as strong candidate negative regulators of nitrate reductase and other nitrogen assimilation-related gene expression in maize in response to changes in nitrogen metabolic balance, and TGA-family transcription factors, which have been identified through systems-level network analysis as important regulatory components of the broader nitrate transcriptional response in Arabidopsis roots (O'Brien *et al*., 2016) [17] (Alvarez *et al*., 2014) [18] (Comparative RNA-Seq analysis, 2016) [28]. Gaudinier *et al*. [21] further demonstrated, through integrated transcriptional and network analysis, that specific transcription factors directly link nitrogen-associated metabolic gene expression to root growth regulation, providing a mechanistic bridge between nitrogen sensing and the architectural plasticity responses long observed physiologically. Hormonal signaling, particularly involving auxin and cytokinin, integrates local and systemic nitrogen status information to coordinate root architectural responses. Local high nitrate supply promotes lateral root elongation through auxin-dependent mechanisms conserved, with some pathway-specific variation, across Arabidopsis and graminaceous species including maize and rice, while systemic nitrogen status is communicated to the root via shoot-derived signals integrated through the transcription factor TCP20 and cytokinin signaling pathways (O'Brien *et al*., 2016) [17] (Sattelmacher *et al*., 1993) [22] (Nadeem *et al*., 2018) [27]. In maize specifically, increased lateral root growth rate under localized nitrate supply has been shown to correlate with elevated local auxin levels in the nitrate-supplied root segments, and the polar auxin transport regulator ZmPIN9 has been implicated in promoting lateral root initiation under locally high nitrate availability in maize, a role analogous to that described for OsNAR2.1 in rice (O'Brien *et al*., 2016) [17] (Sattelmacher *et al*., 1993) [22]. A synthesis of nitrogen-responsive genes, their predicted functions, and associated biological processes relevant to cell-type-specific expression, drawn from the reviewed literature, is presented in Table 4, and associated functional enrichment categories are summarized in Table 5.

Table 4: Nitrogen-Responsive Genes and Regulatory Categories Relevant to Cell-Type-Specific Expression, Synthesized from the Reviewed Literature

Gene / Category	Predicted Function	Associated Biological Process	Representative Sources
NRT1.1 / NPF6.3	Dual-affinity nitrate transceptor	Nitrate sensing, signaling, and transport	[13, 14]
NRT2 family	High-affinity nitrate transport	N uptake under limitation; lateral root initiation signaling	[13, 20]
AMT1 family	Ammonium transport	N uptake; local ammonium-stimulated lateral root emergence	[20]
CPK-NLP module	Calcium-dependent signaling to NLP transcription factors	Central hub linking nitrate sensing to transcriptional response	[16]
LBD transcription factors	Transcriptional regulation of N assimilation genes	Negative regulation of nitrate reductase and related genes	[17, 28]
TGA1/TGA4	bZIP transcription factors	Regulatory components of nitrate transcriptional response	[18]
ZmPIN9 (maize)	Polar auxin efflux carrier	Lateral root initiation under localized high nitrate	[17, 22]

Table 5: Functional Categories and Signaling Pathways Enriched in Nitrogen-Responsive Root Cell-Type Analyses

Functional Category	Representative Pathway/Process	Representative Sources
Nitrate/ammonium transport	High- and low-affinity N transporter systems	[13, 20, 25]
N assimilation metabolism	Nitrate reductase, GS-GOGAT cycle	[16, 25, 33]
Hormonal signaling	Auxin-mediated lateral root elongation; cytokinin systemic signaling	[17, 22, 27]
Transcriptional regulation	LBD, TGA, MYB, bHLH transcription factor activity	[17, 18, 28]
Carbon-nitrogen balance	Sugar transport and glycolysis/TCA integration (e.g., STP4)	[2]
Root developmental plasticity	Lateral root initiation, root hair differentiation, cortex maturation	[1, 2, 20]

Table 6: Directionally Reported Root Phenotypic and Physiological Responses to Nitrogen Deficiency in Maize and Related Cereals

Trait	Reported Directional Response Under N Deficiency	Representative Sources
Lateral root density / foraging response	Increased under mild-moderate deficiency (foraging response)	[20, 22]
Root:shoot biomass ratio	Generally increased, favoring root growth	[20, 26]
Primary root elongation	Variable; species- and severity-dependent	[22, 26]
Chlorophyll content	Decreased under N deficiency	[23]
Photosynthetic N-use efficiency	Can increase per unit leaf N despite reduced biomass	[24]
Overall biomass accumulation	Decreased under sustained/severe deficiency	[23, 26]

Table 7: Comparative Summary of International Studies Utilizing Single-Cell Transcriptomics to Investigate Root Development and Nutrient Stress Responses

Species	Tissue / Focus	Key Finding	Source
Zea mays	Root tips, +/- nitrate	11 cell types; 85 cell type-specific nitrate-response genes identified	Li *et al.* [1]
Zea mays	Root tips, developmental profiling	9 cell types/10 clusters; STP4 identified as cortex hub gene	Bian *et al.* [2]
Zea mays	Root, fungal invasion response	Cell type-specific regulatory networks governing fungal invasion	Cao *et al.* [30]
Arabidopsis thaliana	Whole root, multi-study integration	Organ-scale atlas (110,000+ cells) with developmental trajectories	Shahan *et al.* [8]
Oryza sativa	Root, chromatin + transcriptome	Differentiation trajectories and chromatin accessibility landscape	Liu *et al.* [9]
Arabidopsis thaliana	Root, phosphate limitation	Vascular transcription factors guide epidermal phosphate responses	Wendrich *et al.* [11]

5. Conceptual, Analytical, and Comparative Illustrations

Figure 1 presents the conceptual workflow of single-cell transcriptomic analysis of maize root development under nitrogen deficiency, synthesized from the reviewed literature. Figure 2 provides a schematic of maize root anatomical structure and corresponding scRNA-seq cell populations. Figure 3 presents a representative UMAP visualization of root cell population clustering, illustrating cell-type diversity patterns consistent with the reviewed literature. Figure 4 provides an illustrative cell-type-specific expression pattern of nitrogen transporter and regulatory genes. Figure 5 presents an illustrative pseudotime trajectory of root cell differentiation. Figure 6 synthesizes directionally reported physiological and root architectural responses to nitrogen deficiency. Figure 7 presents an integrative regulatory network linking nitrogen sensing to adaptive root developmental responses.

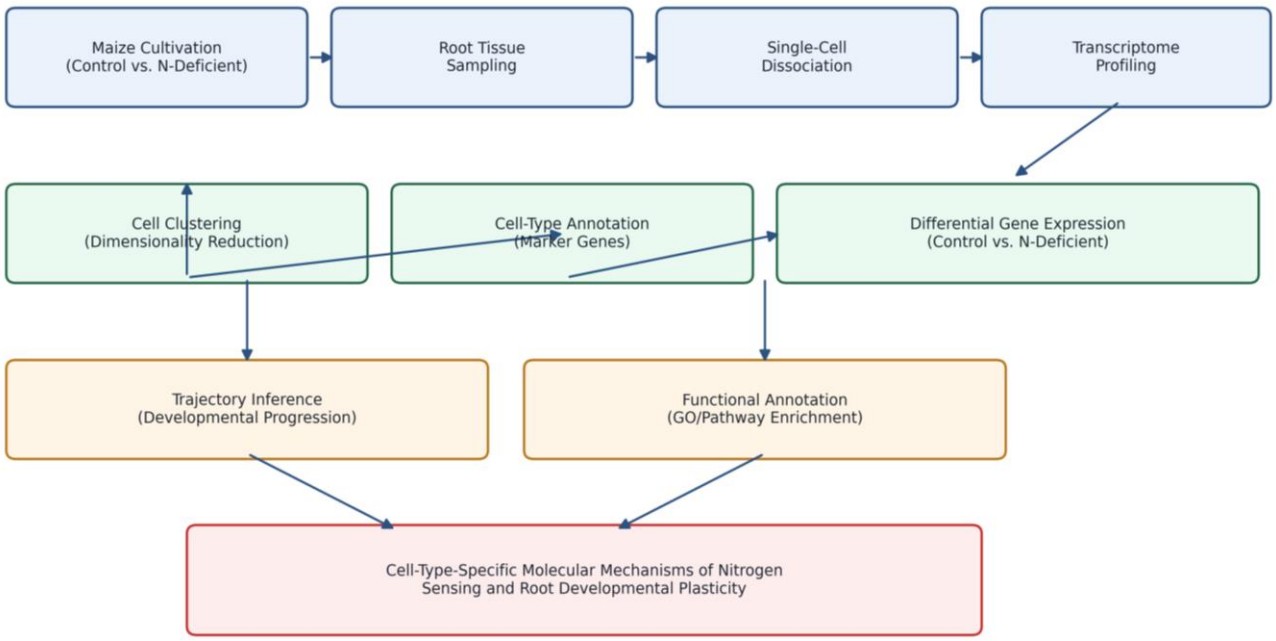

Fig 1: Conceptual workflow of single-cell transcriptomic analysis of maize root development under nitrogen deficiency, synthesized from the reviewed literature.

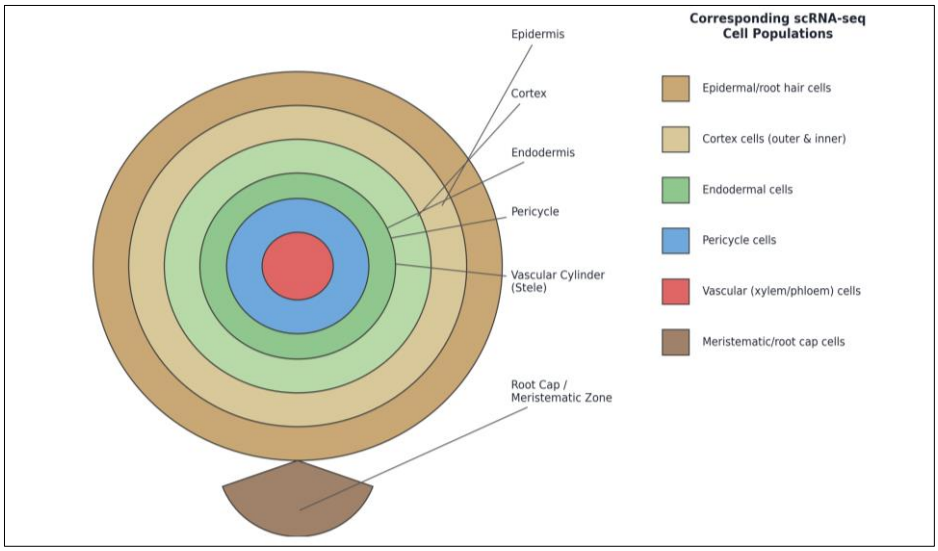

Fig 2: Schematic anatomical structure of the maize root and corresponding cell populations identified by single-cell transcriptomic analysis.

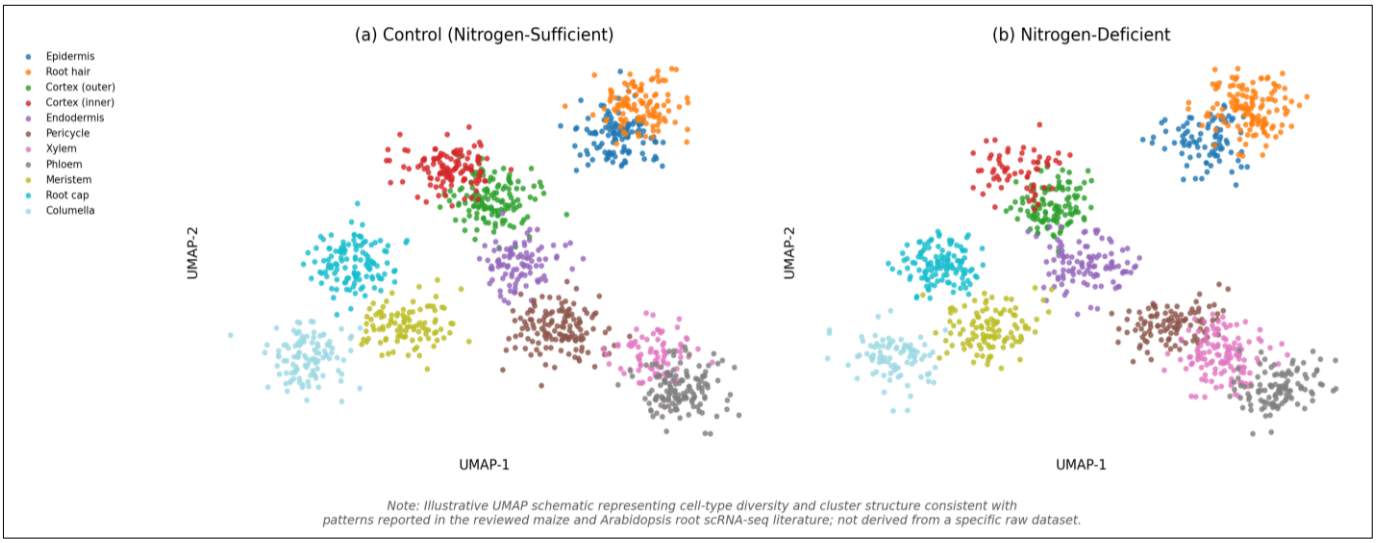

Fig 3: Representative UMAP visualization of root cell population clustering under control and nitrogen-deficient conditions (illustrative schematic; not derived from a specific raw dataset).

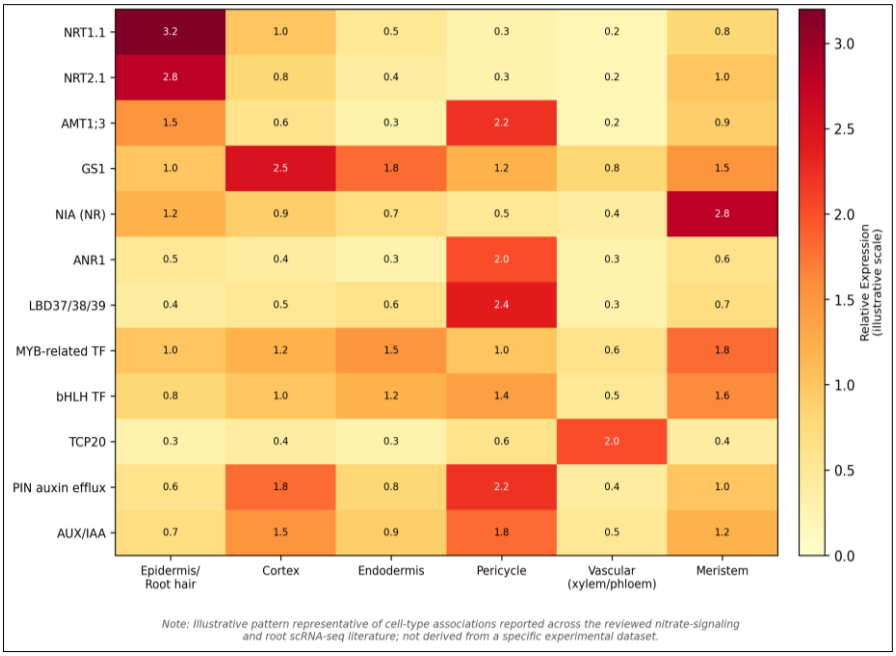

Fig 4: Illustrative cell-type-specific expression pattern of nitrogen transporter, signaling, and regulatory genes across root cell populations (not derived from a specific experimental dataset).

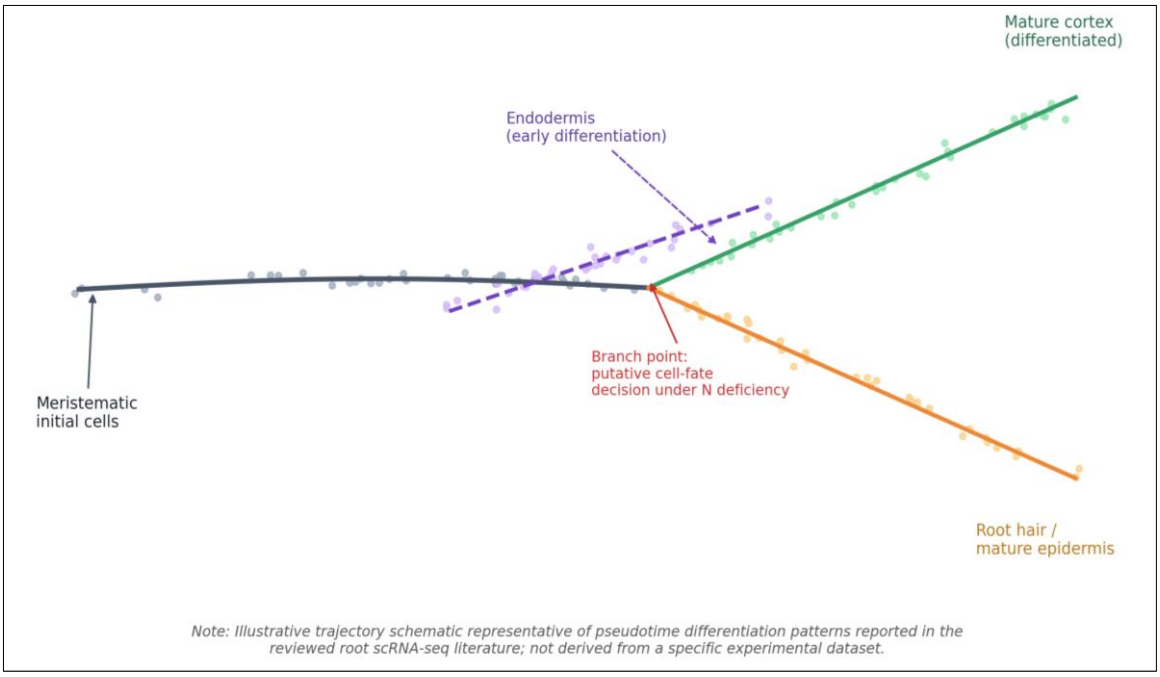

Fig 5: Illustrative pseudotime trajectory of root cell differentiation under nitrogen-deficient conditions (schematic; not derived from a specific experimental dataset).

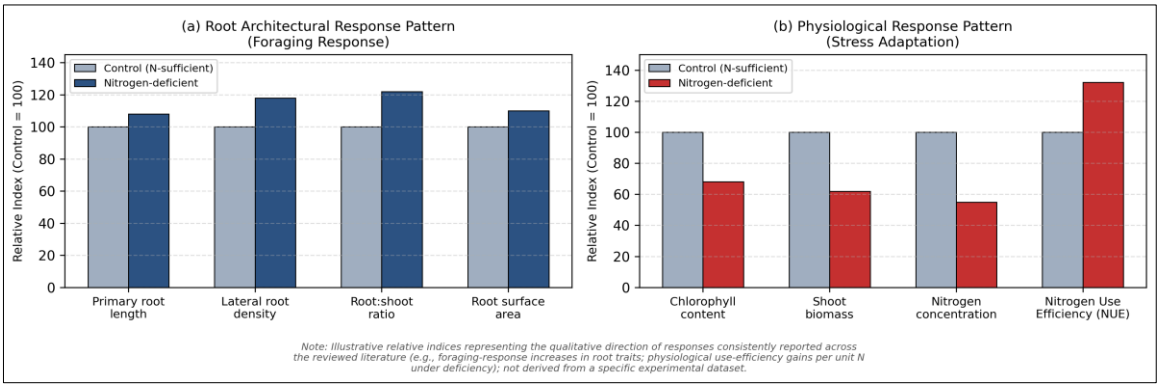

Fig 6: Illustrative directional pattern of root architectural and physiological responses to nitrogen deficiency reported in the reviewed literature.

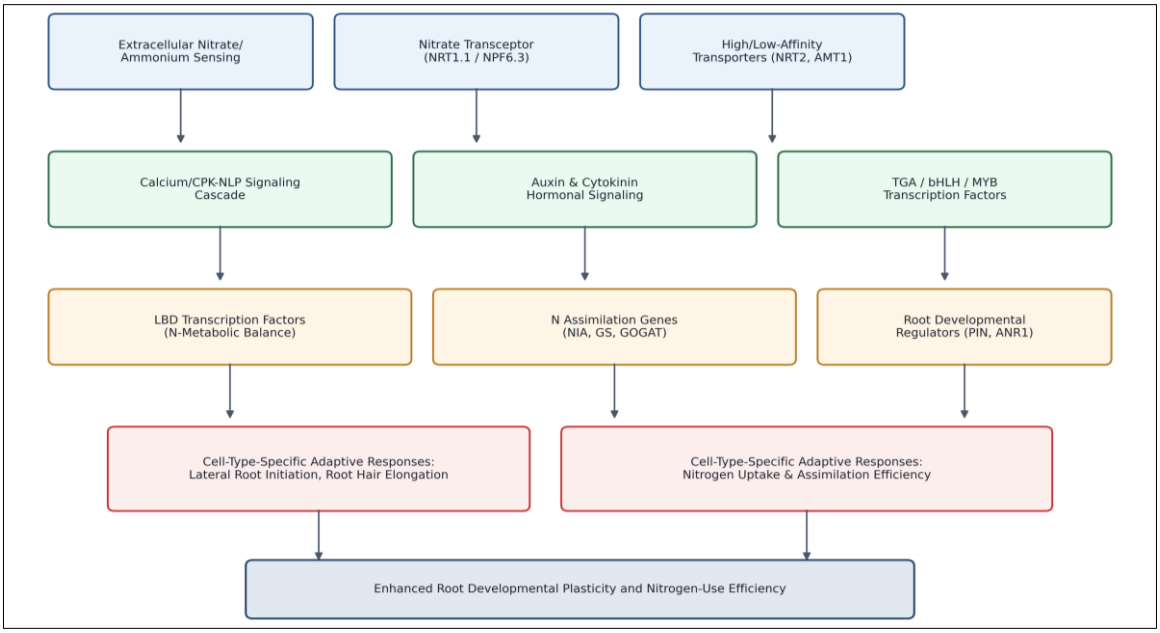

Fig 7: Integrative regulatory network linking nitrogen sensing, signaling, transcriptional regulation, and adaptive root developmental responses in *Zea mays* L.

6. Discussion

The reviewed literature converges on a clear conclusion: nitrogen deficiency responses in maize and other cereal roots are fundamentally cell-type-specific, involving distinct transporter expression, signaling activation, and transcriptional regulatory programs across epidermis, cortex, endodermis, pericycle, and vascular tissue, information that bulk transcriptomic profiling, by design, averages away (Li *et al*., 2022) [1] (Bian *et al*., 2025) [2] (Krouk *et al*., 2010) [13] (Liu *et al*., 2017) [16] (O'Brien *et al*., 2016) [17]. This finding has substantial implications for how nitrogen-use-efficiency research should be conducted going forward: candidate gene and pathway discovery efforts based solely on bulk root transcriptomics risk both false negatives, where a strong but cell-type-restricted signal is diluted below detection in whole-tissue averages, and false positives, where apparent whole-root expression changes actually reflect only a change in the relative proportion of different cell types present rather than a genuine transcriptional response within any single cell type (Li *et al*., 2022) [1] (Bian *et al*., 2025) [2].

Comparison between the extensively characterized Arabidopsis root single-cell literature and the comparatively newer maize-specific literature reveals both encouraging conservation and important divergence. Li *et al*. (Li *et al*., 2022) [1] directly demonstrated conservation of dozens of orthologous cell-type-specific genes between maize and rice root hair, endodermis, and phloem cells, supporting the general principle that fundamental aspects of root cell-type identity and function are evolutionarily conserved across cereals. At the same time, Bian *et al*. (Bian *et al*., 2025) [2] reported that hormone-related gene expression patterns in maize root cell types diverged notably from those previously described in Arabidopsis and rice, a finding that cautions against uncritically extrapolating specific regulatory mechanisms characterized in the Arabidopsis dicot model system directly onto maize without direct empirical verification in the cereal system itself.

The single-cell literature also substantially refines understanding of root developmental plasticity under nitrogen limitation. Rather than a uniform, whole-organ growth response, pseudotime and cell-type-resolved analyses indicate that nitrogen status differentially affects specific developmental transitions, for example, cortex maturation and root hair differentiation from epidermal cell subsets, providing a considerably more mechanistically precise picture of the classical foraging-response phenomenon long described at the whole-root architectural level (Li *et al*., 2022) [1] (Denyer *et al*., 2019) [3] (Jean-Baptiste *et al*., 2019) [4] (Yu *et al*., 2016) [20] (Sattelmacher *et al*., 1993) [22]. This refinement is scientifically valuable because it identifies specific cell populations and developmental transitions as candidate targets for future functional validation, rather than treating root architectural plasticity as a single, undifferentiated trait.

Notwithstanding this progress, an important limitation must be acknowledged directly: the number of published, nitrogen-deficiency-specific single-cell transcriptomic studies conducted directly in maize roots remains small relative to both the broader bulk-transcriptomic nitrogen-response literature in maize and the extensive single-cell nitrate-response literature in Arabidopsis. Li *et al*. (Li *et al*., 2022) [1] remains, as of this review, among the most direct and comprehensive maize-specific single-cell nitrate-response datasets available, meaning that much of the mechanistic detail regarding nitrogen signaling components, NRT1.1-mediated sensing, CPK-NLP signaling, TGA and LBD transcription factor function, is drawn from Arabidopsis studies and inferred, rather than directly demonstrated, to apply with equivalent cell-type specificity in maize (Krouk *et al*., 2010) [13] (Gojon *et al*., 2011) [14] (Liu *et al*., 2017) [16] (Alvarez *et al*., 2014) [18].

Applications in molecular breeding remain at a correspondingly early translational stage. While functional enrichment and gene regulatory network analyses built on single-cell datasets have begun to identify candidate genes and regulatory hubs, such as the STP4 cortex hub gene identified by Bian *et al*. (Bian *et al*., 2025) [2], systematic integration of single-cell-resolved candidate genes with quantitative trait locus mapping, genome-wide association studies, and genomic selection breeding pipelines for maize nitrogen-use efficiency remains limited in the current literature (Gaudinier *et al*., 2018) [21] (Rich-Griffin *et al*., 2020) [32]. This represents a substantial and tractable opportunity, since the QTL and GWAS-based NUE breeding literature in maize is already comparatively mature and could, in principle, be directly cross-referenced against emerging single-cell-resolved candidate gene lists to prioritize functional validation efforts. Publicly accessible maize genomic and functional annotation resources provide an important practical foundation for this cross-referencing effort, offering curated gene models, annotations, and community datasets against which single-cell-resolved candidates can be evaluated (Andorf *et al*., 2016) [31].

Future research priorities emerging from this synthesis include: expansion of direct, nitrogen-deficiency-focused single-cell transcriptomic profiling specifically in maize roots, across multiple genotypes, developmental stages, and nitrogen treatment intensities, to build a dataset comparable in depth to the existing Arabidopsis root nitrate-response literature; systematic cross-referencing of single-cell-resolved candidate genes and regulatory hubs against existing maize NUE QTL and GWAS results to prioritize functional validation; direct empirical testing of the extent to which Arabidopsis-characterized nitrogen signaling mechanisms (NRT1.1 sensing, CPK-NLP signaling, TGA/LBD transcriptional regulation) operate with equivalent cell-type specificity in maize; and continued methodological development to reduce protoplasting-associated expression artifacts and improve capture of rare or protoplasting-resistant cell populations, a technical limitation acknowledged across the plant scRNA-seq literature generally.

7. Conclusion

This review summarizes peer-reviewed research on the study of root development through using single-cell transcriptomics, particularly in reference to *Zea mays* L. The results of the previous studies show that the use of scRNA-seq in the case of the roots of maize is scientifically sound, allowing to identify 9-11 types of root cells and dozens of nitrate-response genes, which could not be identified through the analysis of bulk root tissues. The ability to analyze the levels of expression of nitrogen transporters, the effectiveness of hormonal signaling, and the activity of transcription factors helps to gain a much clearer insight into the process of root architecture under conditions of nitrogen deficiency than provided by the previously performed experiments.

The key result of this review is that the existing research devoted to the use of single-cell transcriptomics in maize faces a lack of studies directly focusing on maize, which means that all the research data has been obtained by making indirect references to Arabidopsis literature.

Researchers are exploring the feasibility of creating maize-centric, nitrogen-deficiency-focused single-cell datasets spanning multiple genotypes and developmental stages and are also investigating the relevance of Arabidopsis-derived nitrogen signaling processes to maize root cell types. For breeders and

crop enhancement programs, identifying candidate genes resolved to the single-cell level and matching them with the existing maize nitrogen-use efficiency quantitative trait loci and genome-wide association study results can help accelerate the implementation of functional single-cell genomics in research and practicality. For agricultural research funding bodies and policy makers, investing in maize-oriented single-cell functional genomics facilities and methodologies is a scientifically justified means of achieving greater nitrogen-use efficiency.

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