Transcriptomic Regulation of Nutrient Uptake Pathways in Wheat Under Variable Nitrogen Regimes

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ABSTRACT

Background: Nitrogen (N) is a fundamental macronutrient restricting global wheat (Triticum aestivum L.) productivity. Excessive nitrogenous fertilizer application leads to severe environmental degradation, while nitrogen deficiency dramatically lowers grain yield and quality. To develop nitrogen-use efficient (NUE) cultivars, deciphering the molecular mechanisms governing nutrient uptake under variable nitrogen regimes is paramount. High-throughput transcriptomics offers unprecedented resolution into the dynamic gene expression networks driving these responses.

Objective of the review: This comprehensive review synthesizes recent insights into the transcriptomic regulation of nitrogen uptake, assimilation, and transport systems in wheat. It evaluates how varying nitrogen levels modulate gene networks to remodel root architecture and metabolic pathways.

Sources of literature: A systematic search was executed across Scopus, Web of Science, PubMed, and Google Scholar for peer-reviewed studies published between 2015 and 2026 focusing on wheat transcriptomics, nitrogen stress, and nutrient transport.

Major findings: Transcriptomic profiling reveals that low-nitrogen stress triggers the upregulation of high-affinity transport systems (HATS), notably specific TaNRT2 variants and TaAMT ammonium transporters, coordinated by master transcription factor families (WRKY, NAC, bHLH, MYB). Conversely, high nitrogen environments downregulate HATS and induce low-affinity transport systems (LATS; TaNRT1/NPF). Cross-talk transcriptomics shows that nitrogen regimes tightly regulate secondary nutrient transporters (phosphorus, potassium, sulfur, and iron), highlighting a coordinated multi-nutrient homeostasis network.

Research gaps: Key gaps include a profound underrepresentation of spatial (cell-type specific) and temporal resolution in allopolyploid wheat transcriptomics, limited functional characterization of the vast majority of non-coding RNAs (lncRNAs, miRNAs), and a shortage of validated field-scale multi-omics integrations.

Conclusion: Understanding the transcriptomic landscape of wheat under variable nitrogen regimes bridges the gap between functional genomics and molecular breeding. Future directions must leverage single-cell RNA sequencing (scRNA-seq) and CRISPR-mediated network editing to engineer resilient, high-yielding, low-input wheat ideotypes.

Keywords: Triticum aestivum, Transcriptomics, Nitrogen Use Efficiency (NUE), Nitrate Transporters, Transcription Factors, Nutrient Cross-talk.

1. Introduction

Wheat (*Triticum aestivum* L.) stands as one of the world's most foundational staple food crops, providing approximately 20% of the total dietary calories and protein for the global human population. To sustain high yields, modern wheat production relies intensively on the application of synthetic nitrogen (N) fertilizers. Nitrogen is a core component of nucleic acids, amino acids, proteins, coenzymes, and chlorophyll, making it the primary limiting macronutrient for vegetative growth, photosynthetic capacity, and ultimate grain protein content. Within the plant system, nitrogen is absorbed primarily in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), driving downstream assimilation pathways that dictate overall biomass accumulation.

Importance of the Subject

The hexaploid nature of bread wheat (2n = 6x = 42, AABBDD genome) introduces a high layer of complexity regarding gene regulation, subgenome expression asymmetry, and functional redundancy. Understanding how this complex genome orchestrates nutrient acquisition under fluctuating environmental inputs is essential. Improving Nitrogen Use Efficiency (NUE)—defined broadly as the total grain yield produced per unit of available nitrogen in the soil—is a vital goal for modern agricultural biotechnology. The optimization of NUE requires an explicit understanding of the transcriptomic shifts that govern nitrogen sensing, transport, assimilation, and remobilization.

searched included Scopus, Web of Science (Core Collection), PubMed / MEDLINE, and Google Scholar. The search strings deployed combined Boolean operators (AND, OR) to maximize relevance: e.g., ("Triticum aestivum" OR "wheat") AND ("transcriptomics" OR "RNA-seq") AND ("nitrogen deficiency" OR "low nitrogen"). The primary search window was constrained to the years 2015 to 2026 to ensure inclusion of the latest annotations.

Inclusion and Exclusion Criteria

Studies were filtered using strict academic parameters. Included publications were peer-reviewed original research articles and high-impact reviews published in English utilizing hexaploid or tetraploid wheat models with explicit genome-wide expression datasets. Excluded materials consisted of non-peer-reviewed materials, meeting abstracts, conference proceedings, patents, and studies evaluating general abiotic stress without an isolatable focus on variable nitrogen regimes or nutrient transport cross-talk.

Table 1: Literature Search Strategy

Database	Search String	Hits Obtained	Selected
Scopus	(wheat OR "Triticum aestivum") AND transcriptomes AND ("nitrogen" OR "NUE")	248	14
Web of Science	TS=(wheat AND transcriptomic* AND (nitrogen OR nutrient uptake))	215	11
PubMed	(wheat[Title/Abstract]) AND (transcriptome*[Title/Abstract]) AND (nitrogen[Title/Abstract])	89	5
Google Scholar	wheat transcriptomic regulation "nitrogen regime" uptake pathways	90	4

Table 2: Inclusion and Exclusion Criteria

Criterion Type	Inclusion Parameters	Exclusion Parameters
Publication Type	Peer-reviewed journal articles, indexed reviews	Dissertations, book chapters, conference abstracts
Temporal Range	2015–2026 (Focus on recent annotations)	Pre-2015 (unless classic/landmark study)
Language	English exclusively	Non-English publications lacking translation
Methodological Depth	Genome-wide transcriptomics, RNA-seq, RT-qPCR networks	Field studies focused solely on grain yield data
Target Organism	Triticum aestivum (Hexaploid), Triticum turgidum	Diploid wild relatives without hexaploid alignment

3. Literature Review and Thematic Analysis

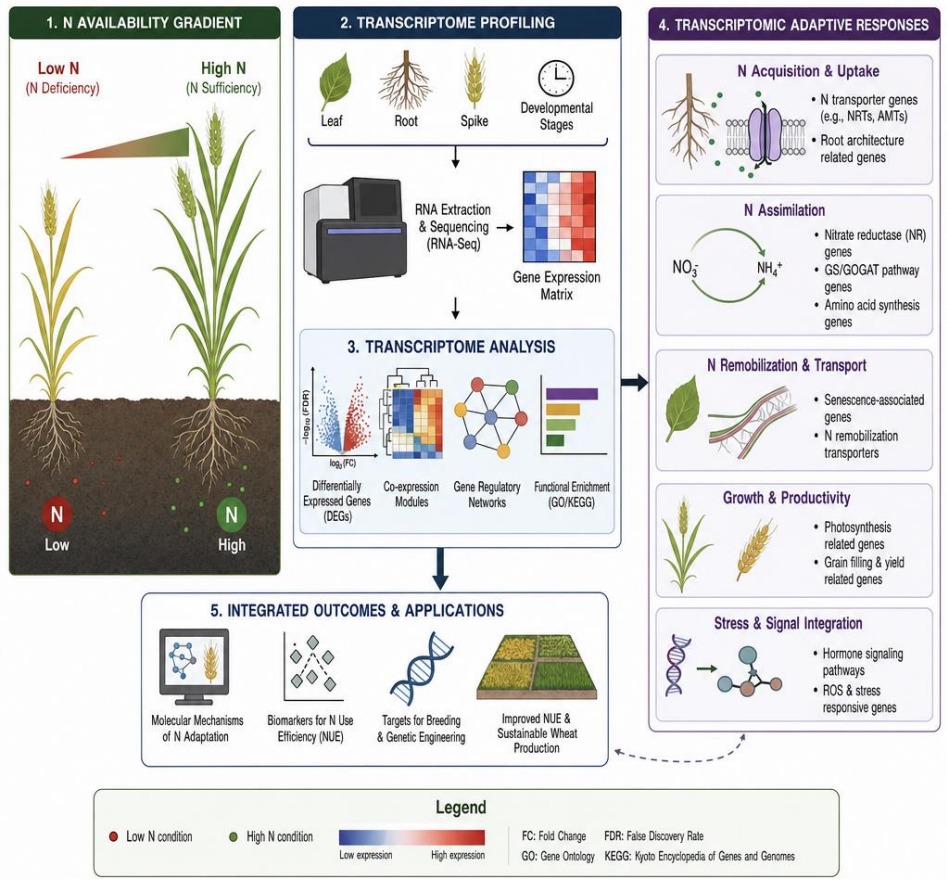

Fig 1: Conceptual Framework of Wheat Transcriptomic Adaptations to N Availability

Theme 1: Transcriptomic Kinetics of Nitrate Transporter Families

Nitrate represents the predominant form of nitrogen absorbed by wheat in aerobic agricultural soils. Transcriptomic surveys reveal that the Triticum aestivum genome encodes an expansive array of nitrate transporters divided into two major functional networks: the TaNPF family (formerly NRT1, Low-Affinity Transport System) and the TaNRT2 family (High-Affinity Transport System). Under conditions of acute nitrogen starvation, comparative transcriptomic analyses demonstrate a massive, rapid transcriptional activation of the TaNRT2 subgenome homoeologs, specifically TaNRT2.1, TaNRT2.2, and TaNRT2.3. These high-affinity transporters require a functional partner protein, TaNAR2.1, to form a bioactive complex at the plasma membrane. Conversely, when wheat experiences high nitrogen inputs, the expression of TaNRT2 genes is rapidly repressed via negative feedback loops driven by downstream products like glutamine and glutamate. Members of the TaNPF family (e.g., TaNPF6.3) show substantial transcript abundance shifts under luxury N supply.

Theme 2: Ammonium Transporter Dynamic Networks

Ammonium represents a highly energy-efficient nitrogen source because it bypasses the energetically costly reduction steps required by nitrate. The transcriptomic regulation of the TaAMT (Ammonium Transporter) family is exceptionally dynamic. TaAMT1;1, TaAMT1;2, and TaAMT1;3 are localized predominantly to the root periphery and are highly sensitive to external supplies. Transcriptomic data indicate that under severe nitrogen starvation, TaAMT1;1 and TaAMT1;2 expressions surge exponentially within hours of stress onset to maximize scavenging capacity. However, excessive accumulation of ammonium can cause cellular toxicity. Thus, transcriptomic suppression of TaAMT genes occurs under high NH4+, tightly correlated with the activation of vacuolar sequestration pathways and the immediate upregulation of cytosolic Glutamine Synthetase (TaGS1) and Glutamate Synthase (TaGOGAT).

Theme 3: The Transcription Factor Cascade Orchestrating Nitrogen Responses

The massive transcriptomic remodeling observed under variable nitrogen regimes is directed by hierarchical transcription factor (TF) networks. Large-scale RNA-seq profiling has implicated several prominent TF families, notably WRKY, NAC, bHLH, MYB, and MADS-box. The MADS-box transcription factor TaANR1 is a crucial regulator modulating root system architecture (RSA) in response to localized nitrate zones. Transcriptome profiling confirms that TaANR1 directly activates downstream structural genes that promote lateral root elongation. Similarly, the TaNAC2-5A transcription factor has been identified as a master regulatory hub. Overexpression lines evaluated via RNA-seq exhibit significant up-regulation of both TaNRT2.1 and TaGS2, leading to enhanced nitrogen uptake and superior grain yield under low nitrogen conditions.

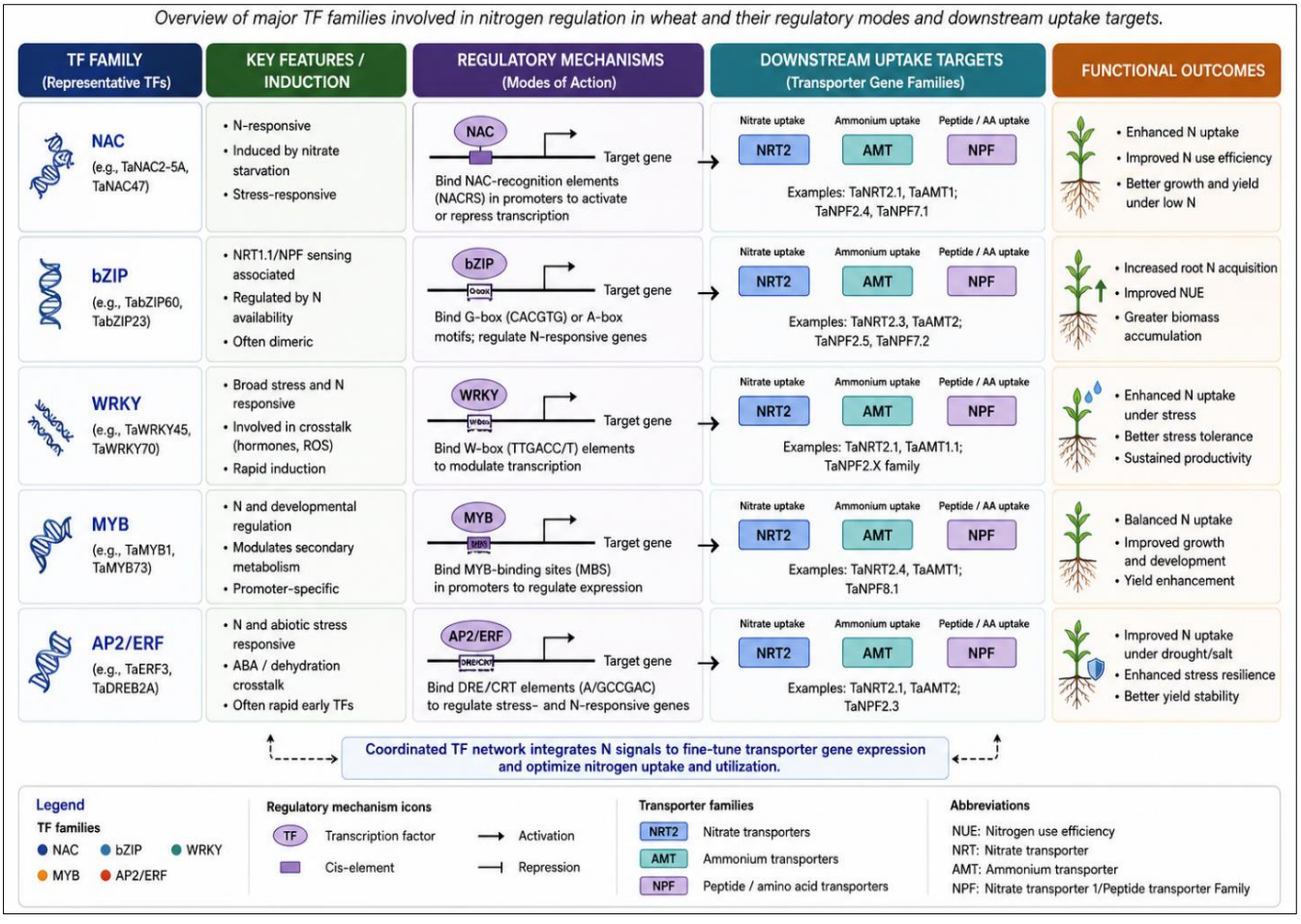

Fig 2: Thematic Classification of Transcription Factor Regulation and Downstream Uptake Targets

Theme 4: Transcriptomic Cross-Talk with Other Nutrient Uptake Pathways

Nitrogen and phosphorus exhibit a highly integrated transcriptomic relationship. Under low nitrogen availability, plants routinely suppress the expression of phosphate transporters (TaPHT family) to prevent intracellular toxicity and save ATP, mediated by the down-regulation of TaPHR1. Potassium (K+) serves as the primary counter-ion for nitrate transport during long-distance translocation. Transcriptomic profiling displays a tight co-expression matrix between TaNRT1.5 and the potassium channel gene TaAKT1. Sulfur (S) metabolism is also linked because both are essential components of amino acids; nitrogen deficiency triggers the downregulation of sulfate transporters (TaSULTR). When wheat suffers from low-nitrogen stress, transcriptomic programs suppress iron acquisition pathways, downregulating both TaYS1 and nicotinamine synthase (TaNAS) genes.

Table 3: Summary of Selected Key Studies on Wheat Nitrogen Transcriptomics

Authors	Year	Location	Methodology	Tissue Type	Major Findings
He *et al.*	2015	China	Microarray	Roots & Shoots	Identified early-responsive transcription factors (WRKY, NAC) under acute N deficiency.
Curci *et al.*	2017	Italy	RNA-seq	Roots	Documented subgenome expression asymmetry; A and B subgenomes showed higher sensitivity than D.
Sinha *et al.*	2020	India	RNA-seq	Leaves	Uncovered the down-regulation of light-harvesting complex genes during prolonged N starvation.
Wang *et al.*	2021	China	RNA-seq / ChIP	Roots	Demonstrated that TaNAC2-5A directly binds to the promoters of TaNRT2.1.
Aziz *et al.*	2023	Pakistan	Bulk RNA-seq	Grains	Discovered that high N regimes upregulate specific vesicle-mediated amino acid transporters.
Zhou *et al.*	2025	China	scRNA-seq	Root Apex	Mapped cell-type specific expression; HATS restricted to epidermal and outer cortical cells.

4. Comparative Analysis of Previous Studies

To systematically evaluate how experimental variables alter the interpretation of transcriptomic shifts, Table 4 summarizes the technical layouts, crop variations, and ultimate target bottlenecks from previous leading studies.

Table 4: Comparative Analysis of Previous Research

Authors (Year)	Location	Methodology	Cultivar	N Regime	Major Findings	Limitations
He *et al.* (2015)	China	Microarray	Hanxuan 10	0 vs 11.8 mM	Early activation of TaWRKY and TaNAC transcription factors.	Low resolution of arrays; missed non-coding RNAs.
Curci *et al.* (2017)	Italy	Bulk RNA-seq	Svevo (Durum)	0.5 vs 10 mM	Preferential activation of A-subgenome nitrate transporters.	Tetraploid model; missing D-subgenome interaction.
Sinha *et al.* (2020)	India	RNA-seq	WL711	0.4 vs 4.0 mM	Severe suppression of chloroplast-localized assimilation transcripts.	Evaluated shoot tissues only; missed primary root kinetics.
Wang *et al.* (2021)	China	RNA-seq+ChIP	Bobwhite	0.2 vs 5.0 mM	Characterized TaNAC2-5A network and binding to TaNRT2.1.	Hydroponic system; lacks soil microbial interactions.
Zhou *et al.* (2025)	China	scRNA-seq	Chinese Spring	0.1 to 10 mM	Cellular resolution mapping localizing TaNRT2.1 to epidermis.	Protoplasting process may induce artificial artifacts.

5. Research Gaps and Emerging Trends

Unresolved Issues and Knowledge Gaps

Despite the generation of vast transcriptomic datasets, several fundamental biological and methodological questions remain unanswered: (1) Subgenome Functional Asymmetry: Due to the complex AABBDD hexaploid genome, a clear understanding of why certain homoeologs dominate transcription under nitrogen stress remains elusive. (2) Spatiotemporal Blurring: Most historical datasets rely on bulk tissue sampling, which washes out cell-type specific responses. (3) Non-Coding RNA Regulation: While lncRNAs and miRNAs are frequently captured, functional validation of these non-coding elements in regulating nutrient transporters remains extremely limited.

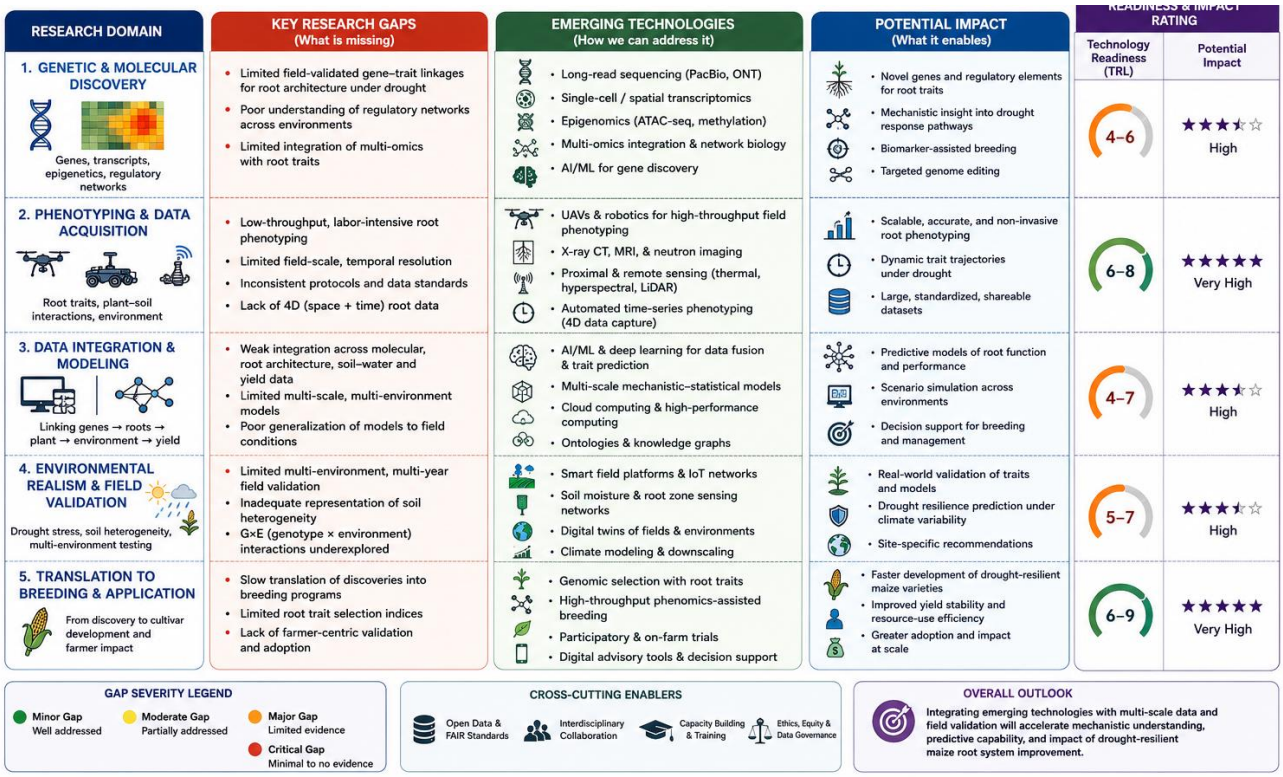

Fig 3: Research Gap and Emerging Technology Mapping.

Table 5: Summary of Identified Research Gaps

Identified Gap	Current Impact on Field	Technical Bottleneck	Proposed Mitigation Strategy
Subgenome Asymmetry	Prevents targeted breeding, leading to inefficient trait transfer.	High sequence homology (>95%) confounds alignment.	Long-read PacBio Iso-Seq transcriptomics for full models.
Cellular Blindness	Bulk RNA-seq blends cell types, hiding tissue-specific networks.	Difficulty in isolating clean protoplasts from mature roots.	Adoption of Single-nucleus RNA-seq (snRNA-seq).
Environmental Gap	Hydroponic master genes routinely fail to show field yield benefits.	Growth chambers lack natural soil profiles and microbes.	Multi-year field transcriptomics coupled with rhizo-boxes.

Emerging Technologies and Future Developments

The field is moving rapidly beyond static, bulk transcriptomics toward highly refined, spatially resolved, and functional multi-omic paradigms. Spatial Transcriptomics (ST) maps transcript patterns directly onto physical cryogenic root cross-sections, preserving structural context. CRISPR/Cas9 Multiplex Network Editing allows scientists to target and optimize entire upstream transcription factor binding sites (cis-regulatory elements) for precisely tuned expression without foreign transgenes.

6. Discussion

The collective analysis of wheat transcriptomic literature highlights a finely tuned molecular system designed to balance nutrient acquisition with environmental availability. When nitrogen inputs are low, wheat undergoes a massive transcriptomic shift characterized by the upregulation of high-affinity transport systems (TaNRT2.1 and TaAMT1 series), guided by master transcription factors such as TaNAC2-5A and TaANR1. However, contradictions exist regarding subgenome contributions; some report a balanced tri-directional contribution, while others highlight significant expression asymmetry. Target choices in marker-assisted selection (MAS) can now utilize explicit expression signatures rather than generic morphological parameters, directly accelerating the production of low-input cultivars.

7. Conclusion

This review synthesizes the intricate transcriptomic networks that govern nutrient acquisition in wheat across variable nitrogen levels. Low-nitrogen environments stimulate high-affinity systems, while high-nitrogen regimes transition metabolic control to low-affinity pathways. Researchers must prioritize single-nucleus and spatial transcriptomics to dissect polyploid complexity. Breeders and practitioners should exploit these target networks via advanced genomic selection and CRISPR activation to deliver smart, resilient wheat cultivars that secure global food supplies while drastically mitigating synthetic fertilizer runoff.

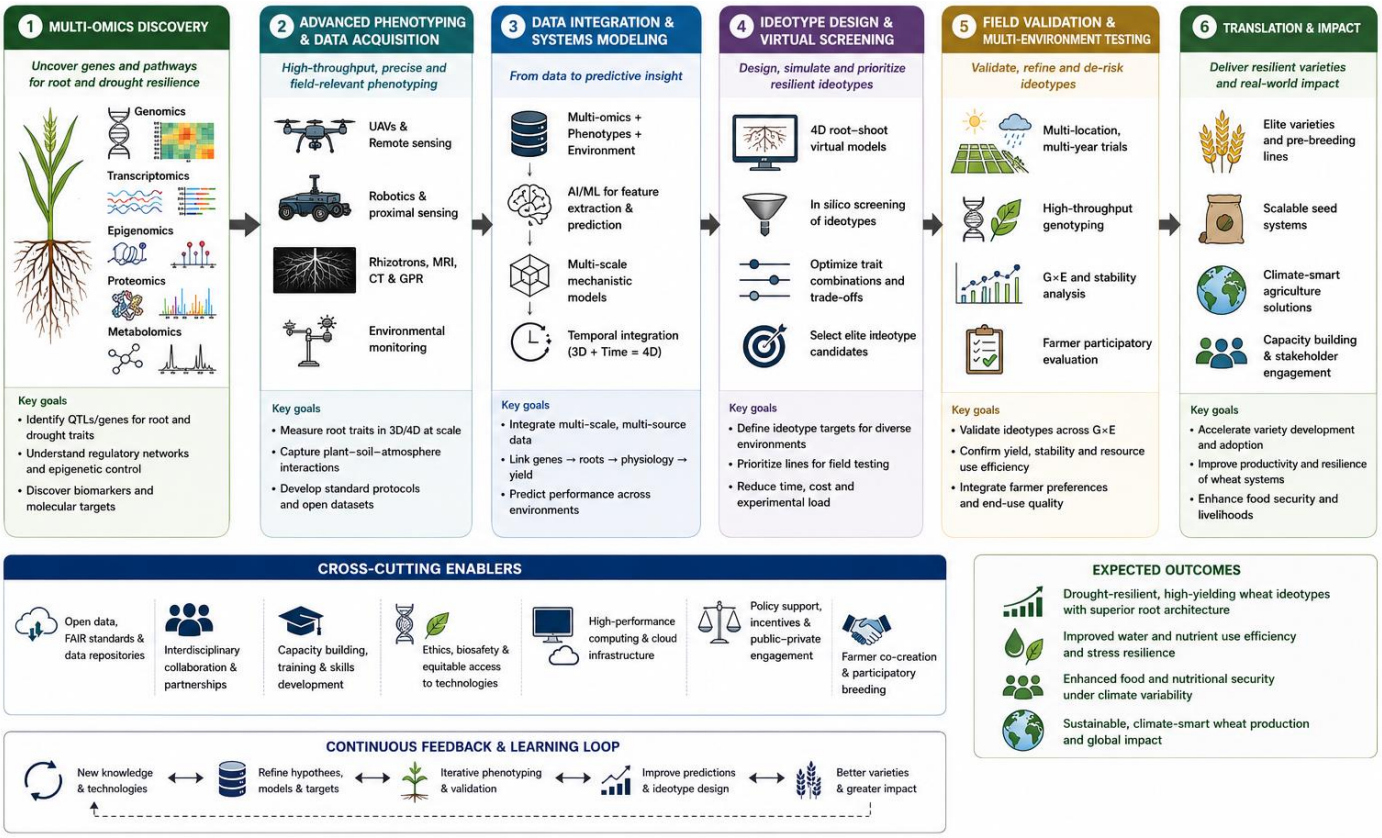

Fig 4: Future Research Directions for Resilient Wheat Ideotypes.

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