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

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Received: 13 Dec 2022
Revised: 05 Jan 2023
Accepted: 27 Jan 2023
Published: 17 Feb 2023

E-ISSN: 2989-5359
DOI: https://doi.org/10.54660/ejsa.2023.3.1.63-73

ABSTRACT

Background: Nitrogen (N) is a crucial nutrient for the growth of wheat (*Triticum aestivum* L.) and its exploration, transportation and recycling is regulated by various transcriptional programs reacting timely to the changing nitrogen conditions in soil. The last decade has seen the amazing increase in transcriptomic data regarding the mechanisms of nitrate, ammonium, phosphorus, and potassium transport due to the implementation of RNA-seq technology and genome-wide expression profiling methods.

Objective: This paper provides a comprehensive review of transcriptomic data on regulatory mechanisms of nutrient uptake in wheat under different nitrogen availability conditions, focusing on biosynthesis and regulation of nitrate (NPF, NRT2) and ammonium (AMT) transporters, as well as nitrogen-responsive transcription factors (NLP, NAC, bZIP, MYB, WRKY, WFZP) and their interconnections with the phosphorus and potassium sources. Please note that major finding of the review are to be announced. When analyzing transcriptome data, we find out that there is a dependence of the expression of NPF and NRT2 genes on the cultivar and transcription factors from the NLP and NAC families have a major role in their regulation. The AMT1 gene family should also have high-affinity transporter system. The existing gap in research is that due to variability in experimental design, nitrogen rate, growth stage or genome reference, results cannot be compared from one study to another. Thus, integrated approach should be utilized in the breeding of nitrogen efficient varieties.

Keywords: *Triticum aestivum*, nitrogen use efficiency, transcriptomics, nitrate transporter, ammonium transporter, gene expression, nutrient signaling, RNA sequencing

1. Introduction

Wheat (*Triticum aestivum* L.) is grown on over 220 million hectares across the globe and was recently providing almost one-fifth of human calories, which makes it vital for food security all around the world. Among all minerals required by wheat, nitrogen (N) has the greatest impact on biomass production, tillering, grain yield, and the level of protein in grain, which is why it is used in huge doses in comparison to other fertilizers in a cereal crop. The total production of cereals worldwide has been steadily increasing by about 3.4 times over the last 60 years, mostly due to the rise in nitrogen fertilizer use by around 10 times in cereal farming [24, 28]. However, the nitrogen use efficiency (NUE) in wheat production which is usually understood as crops produced with the utilization of 1 kg of nitrogen does not exceed 50% in many cases [24, 26, 29], and thus results in considerable losses of nitrogen through leaching, denitrification, and volatilization that contribute to eutrophication, groundwater pollution, and greenhouse gas emissions.

At the base of NUE are molecular processes regulating nitrogen uptake, assimilation, transport, and remobilization, and all of these processes are regulated transcriptionally. Wheat roots primarily absorb nitrate (NO3-) and ammonium (NH4+), and their uptake is highly dependent on various gene families that have evolved the capability of nitrogen extraction for functional diversification: NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER FAMILY (NPF), NITRATE TRANSPORTER 2 (NRT2), and AMMONIUM TRANSPORTER (AMT) families [1-3]. Transporter genes do not act on their own; the expression of transporter genes is controlled by nitrate- and ammonium-responsive transcription factors, including NIN-LIKE PROTEIN (NLP), NAC-domain, bZIP, MYB, and WRKY transcription factors, which act as intermediaries to blend the external nitrogen status with internal developmental and metabolic signaling processes [6, 9, 10]. With nitrogen status in agricultural soils inherently variable - depending on fertilizers, soil hydration, temperature, and activity of nitrogen-fixing bacteria - wheat possesses transcriptional mechanisms permitting modifications of nutrient uptake pathways in a matter of hours [4, 5].

The modern RNA sequencing technology also known as Next Generation Sequencing (NGS) and associated with the publication of the annotated hexaploid wheat genome allowed to conduct a genome-wide investigation of the mentioned gene families and their expression patterns in different tissues, stages of development, and nitrogen regimes [1, 3]. An increasing number of research works in comparative transcriptomics allow describing a variety of wheat genotypes characterized by distinct NUE levels and origins, involving the analyses of the expressions of certain genes in plants grown under limited and sufficient nitrogen levels

and resulting in identification of DEGs involved in the nitrogen response related to transporter family, transcription factors, and kinases activity as well as the role of microRNAs in the regulation of nitrogen response [4-7, 9].

The objective of this review is to: (i) gather transcriptomic information on the gene families that transport nitrate and ammonium in wheat under multiple nitrogen conditions; (ii) analyze the transcription regulation system which comprises transcription factors and epigenetic regulators that control how genes associated with nitrogen use are expressed; (iii) study the connections between nitrogen signaling and the transcription of the genes that participate in the transport of phosphorus and potassium; (iv) determine problems in methodology and knowledge in reviews mentioned; and (v) list modern technological methods that can be used to convert transcriptomic knowledge into more efficient wheat varieties. This review is limited to transcriptomic studies on hexaploid bread wheat and tetraploid durum wheat that have been published from 2015 to 2025, with some literature published before this in some cases when necessary.

2. Methodology of Literature Review

2.1 Literature Search Strategy

A structured literature search was conducted across Scopus, Web of Science, PubMed/PMC, Google Scholar, and the preprint/repository platforms bioRxiv and Frontiers, covering the period January 2015 to June 2025. Search strings combined controlled vocabulary and free-text terms including: ("wheat" OR "*Triticum aestivum*") AND ("nitrogen" OR "nitrate" OR "ammonium") AND ("transcriptome" OR "RNA-seq" OR "gene expression" OR "differentially expressed genes"), with subsidiary searches incorporating "nitrate transporter", "NRT", "NPF", "AMT", "nitrogen use efficiency", "phosphate transporter", and "potassium transporter". Reference lists of retrieved articles and relevant review papers were hand-searched to identify additional eligible studies (snowballing). Table 1 summarizes the search strategy.

Table 1: Literature search strategy

Element	Detail
Databases searched	Scopus; Web of Science; PubMed/PMC; Google Scholar; Frontiers repository
Core search terms	wheat, *Triticum aestivum*, nitrogen, nitrate, ammonium, transcriptome, RNA-seq, differentially expressed genes, nitrate transporter, NRT, NPF, AMT, nitrogen use efficiency
Supplementary terms	phosphate transporter, potassium transporter, transcription factor, NLP, NAC, WRKY, microRNA, root architecture
Search period	January 2015 - June 2025 (foundational pre-2015 studies cited for mechanistic context)
Search restriction	English language; peer-reviewed journal articles and indexed preprints
Supplementary sources	FAO statistical reports; global nitrogen-use-efficiency meta-analyses

2.2 Inclusion and Exclusion Criteria

Studies were included if they reported original transcriptomic, genome-wide expression, or functional-genomic data on nitrogen-, phosphorus-, or potassium-responsive gene families in wheat, were published in a peer-reviewed journal (or an indexed, subsequently peer-reviewed preprint), and were available in English. Studies were excluded if they were duplicate records across databases, non-peer-reviewed opinion pieces or non-academic sources, focused exclusively on species other than wheat without a comparative wheat component, or reported only agronomic/field-yield data without any molecular or transcriptomic component. Table 2 details the inclusion and exclusion criteria.

Table 2: Inclusion and exclusion criteria

Criterion type	Included	Excluded
Publication type	Peer-reviewed original research articles, systematic reviews, indexed conference-derived journal papers	Non-peer-reviewed blogs, theses without journal publication, conference abstracts only
Species focus	*Triticum aestivum* (bread wheat), Triticum durum (durum wheat); comparative studies including wheat	Studies exclusively on non-wheat species with no wheat comparator
Data type	RNA-seq, microarray, qRT-PCR-validated transcriptomic, genome-wide gene family/expression analyses	Purely agronomic/yield trials with no gene expression or transcriptomic component
Language	English	Non-English without verified translation
Time frame	2015-2025 (with landmark earlier studies for background)	Superseded/duplicate records already captured via database overlap

2.3 Study Selection Process

The study selection process followed a PRISMA-style workflow. An initial pool of records was identified through database searching, after which duplicate records were removed. Titles and abstracts were screened for topical relevance to nitrogen-responsive transcriptomics in wheat, followed by full-text assessment against the inclusion and exclusion criteria in Table 2. Studies retained after full-text review form the evidence base synthesized in Section 3. Figure 1 presents the PRISMA-style flow diagram summarizing this process.

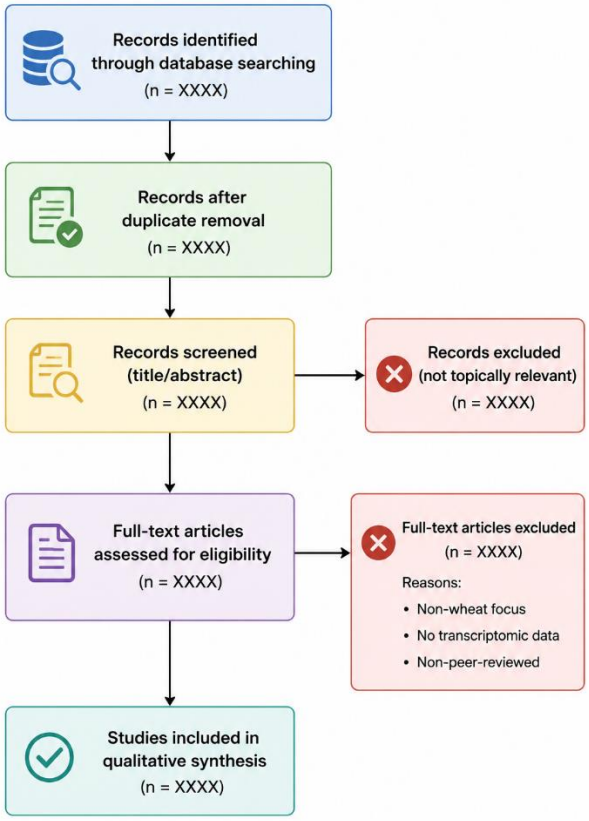

Fig 1: PRISMA-style flow diagram of the literature selection process

The final evidence base spans genome-wide gene-family characterizations, comparative RNA-seq studies of contrasting-NUE cultivars, transcription-factor functional studies, and meta-analyses of global nitrogen fertilization efficiency, allowing thematic synthesis across transporter biology, regulatory networks, and agronomic context (Table 3). Figure 2 illustrates the approximate distribution of the reviewed transcriptomic literature by publication year, reflecting the marked increase in wheat nitrogen-transcriptomics output following release of the annotated hexaploid reference genome in 2018.

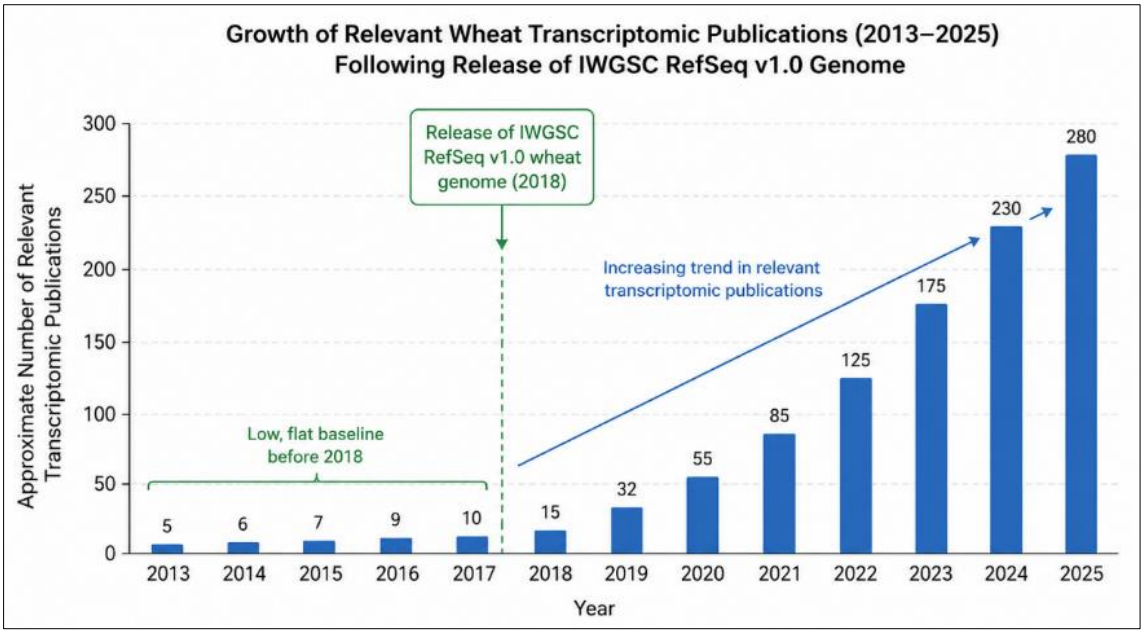

Fig 2: Approximate publication trend of wheat nitrogen-transcriptomics literature, 2013-2025

3. Literature Review and Thematic Analysis

3.1 Nitrate Transporter Gene Families: NPF and NRT2

The NPF and NRT2 gene families constitute the principal molecular machinery for nitrate acquisition in wheat. Genome-wide characterization of the complete wheat NPF family identified 331 NPF genes clustering into 113 homoeologous groups and eight phylogenetic subfamilies, with expression profiling across root, leaf, stem, node, spike, and grain tissue revealing at least six distinct expression-response patterns to nitrogen supply and developmental stage

[1]. A substantial proportion of root-expressed NPF genes showed stable, constitutive expression irrespective of nitrogen fertilization, suggesting a housekeeping role in nitrogen homeostasis and root growth, while a smaller, functionally distinct subset was strongly up-regulated under high nitrogen supply or at specific developmental stages, implicating these genes specifically in nitrate-responsive uptake and translocation [1, 2]. In durum (tetraploid) wheat, genome-wide characterization identified 211 NPF and 20 NRT2 genes sharing overlapping cis-regulatory elements and transcription-factor binding sites, indicative of a coordinated, partially shared regulatory architecture governing nitrate transport and utilization; NRT2 family members further separated into two co-expression modules, one root-expressed and NAR2/NRT3-dependent, and a second induced in reproductive tissues during grain and anther maturation [3].

Comparative transcriptomic analysis of nitrogen-efficient and nitrogen-inefficient Indian wheat cultivars found markedly greater numbers of differentially expressed genes in the efficient genotype (1,653 DEGs, split between root and shoot tissue) relative to the inefficient genotype (753 DEGs), with nitrate transport, nitrogen-compound metabolism, and glutamine biosynthesis pathways enriched among up-regulated genes in the efficient cultivar [4]. This finding is broadly consistent with an independent RNA-seq study of three Australian bread wheat cultivars profiled at anthesis and during grain filling, which reported over 12,000 nitrogen-responsive DEGs and identified low-affinity nitrate transporter (NRT1.1) and high-affinity nitrate transporter transcripts among the most strongly regulated genes, though the direction of regulation for NRT1.1 differed between studies - decreasing under nitrogen stress in the Australian cultivars, in contrast to genotype-dependent induction patterns reported elsewhere [4, 5]. Such discrepancies likely reflect differences in developmental stage sampled (vegetative versus post-anthesis), cultivar genetic background, and the precise nitrogen-dose threshold used to define "low" versus "sufficient" nitrogen, underscoring the difficulty of direct cross-study comparison in this literature.

A weighted gene co-expression network analysis (WGCNA) integrating 47 wheat transcriptome datasets under low-nitrogen stress identified 8,468 differentially expressed genes overall, with roots and shoots displaying largely opposite regulatory patterns: the majority of root DEGs were up-regulated whereas the majority of shoot DEGs were down-regulated under low nitrogen [6]. Gene ontology enrichment confirmed that nitrate reductase activity, nitrate assimilation, and broader nitrogen-compound metabolism were significantly enriched in both tissues, reinforcing the centrality of nitrate transport and assimilation machinery to the whole-plant low-nitrogen response [6]. Collectively, these NPF/NRT2 studies agree that nitrate transporter gene expression is tissue-specific, developmentally dynamic, and genotype-dependent, but disagree on the precise magnitude and direction of regulation for individual isoforms - a inconsistency attributable in large part to methodological heterogeneity rather than biological contradiction per se.

3.2 Ammonium Transporter (AMT) Family Regulation

Ammonium is agronomically important as the dominant nitrogen form in acidic or waterlogged soils where nitrification is suppressed, and its uptake in wheat is mediated by the AMT gene family. Genome-wide identification studies have characterized 23 TaAMT genes in wheat, revealing both tissue-specific and constitutive expression patterns, promoter cis-regulatory elements linked to hormone signalling and abiotic-stress adaptation, and expression responsiveness to varying ammonium nitrogen supply as well as to pathogen challenge (stripe rust and powdery mildew) [7, 8]. Functional characterization confirmed that TaAMT1;1a, TaAMT1;1b, and TaAMT1;3a localize to the plasma membrane and complement ammonium-uptake-deficient yeast and Arabidopsis mutants, verifying their role as bona fide high-affinity ammonium transporters; nitrogen deprivation induced expression of all three genes in roots and leaves, while ammonium resupply rapidly and specifically suppressed TaAMT1;3a without altering TaAMT1;1a or TaAMT1;1b transcript levels [8]. This isoform-specific responsiveness mirrors the post-translational, phosphorylation-based feedback loop described for AtAMT1.1 and AtAMT1.3 in Arabidopsis, in which low-nitrogen conditions activate transporter activity while high-nitrogen conditions promote transporter degradation, suggesting partial conservation of ammonium-sensing mechanisms between model and crop species [7].

Comparatively fewer wheat transcriptomic studies have targeted the AMT family relative to nitrate transporters, and the interaction between ammonium and nitrate transporter regulation - relevant given that most agricultural soils present a mixed nitrate-ammonium nitrogen pool - remains comparatively underexplored in wheat specifically, though better characterized in rice and Arabidopsis. This represents a clear asymmetry in the literature that constrains holistic understanding of nitrogen-form-specific transcriptional regulation in wheat.

3.3 Transcriptional Regulators of the Nitrogen Response: NLP, NAC, bZIP, MYB and WRKY Networks

Transcription factors integrate nitrate and ammonium sensing with downstream transporter gene expression. In Arabidopsis, NIN-LIKE PROTEIN (NLP) family transcription factors, particularly NLP6 and NLP7, were established as central, nitrate-responsive master regulators that bind the nitrate-responsive cis-element and whose activity is post-translationally modulated by nitrate signalling itself [9, 10]. Genome-wide expression analysis of wheat NLP genes identified 18 TaNLP members grouped into three phylogenetic clades, with expression profiling confirming their responsiveness to nitrate-nitrogen stress, supporting a conserved regulatory role for this family in cereal nitrogen signaling [11]. Downstream of NLP-type regulation, wheat-specific NAC-domain transcription factor TaNAC2 was shown to directly bind the promoter of the NRT2-type transporter gene TaNRT2.5 and positively regulate its expression; overexpression of TaNAC2-5A increased grain nitrate concentration, seed vigour, and yield, while RNA interference of TaNRT2.5 produced the opposite phenotype, establishing a direct transcription-factor-to-transporter regulatory module with demonstrated agronomic consequence [12].

A contrasting regulatory logic was reported for the bZIP-family transcription factor TabZIP60, whose expression is repressed by nitrate resupply in nitrogen-deprived wheat; RNA-interference knockdown of TabZIP60 increased NADH-dependent glutamate synthase (NADH-GOGAT) activity, lateral root branching, nitrogen uptake, and spike number, improving grain yield by more than 25% under field conditions, whereas TabZIP60 overexpression had the opposite, yield-suppressing effect [13]. This indicates that not all nitrogen-responsive transcription factors act as positive

regulators of NUE - TabZIP60 functions as a negative regulator of NADH-GOGAT and, by extension, of nitrogen assimilation efficiency - a nuance sometimes obscured when nitrogen-responsive genes are collectively framed as "NUE-promoting" candidates without regard to the sign of their regulatory effect.

Root architecture and nitrogen uptake efficiency are further coordinated at the chromatin level. The class II AP2/ERF transcription factor WHEAT FRIZZY PANICLE (WFZP) was found to interact with the chromatin-remodelling complex subunit TaSYD; wfzp and Tasyd mutants both displayed reduced lateral root number, root length, and nitrogen uptake efficiency, and the WFZP-TaSYD module was shown to promote expression of root-development and nitrate-uptake-related genes by modulating chromatin accessibility and histone modification [14]. This finding extends the regulatory picture beyond sequence-specific transcription factors to epigenetic and chromatin-remodelling mechanisms, a dimension that comparative transcriptomic studies focused solely on DEG counts and gene ontology enrichment do not typically capture. Broader transcription-factor family surveys under low-nitrogen stress additionally implicated MYB-related genes (38 of 38 family members differentially expressed) functioning in a markedly tissue-specific manner, alongside WRKY, zinc-finger, and RING-finger transcription factors co-expressed with nitrate transporter, amino-acid transporter, and sugar-transporter genes in the high-NUE cultivar PBW677 [4, 6]. Taken together, the transcription-factor literature converges on a hierarchical model - NLP-family sensors acting upstream, NAC/bZIP/AP2-ERF factors providing intermediate, often genotype- and tissue-specific regulation, and chromatin remodellers modulating accessibility - while diverging on the precise identity of the dominant hub regulators, which appear to vary by cultivar, growth stage, and tissue.

3.4 Root Architecture, Nitrogen Signalling Crosstalk, and Whole-Plant Coordination

Root system architecture strongly conditions nitrogen acquisition, and NRT1.1 itself functions dually as a nitrate sensor ("transceptor") and transporter that influences root and shoot growth relative to nitrogen availability [15]. Reviews of cereal NUE improvement emphasize that root hair density and length augment root surface area and nitrate/ammonium ion interception, and that ammonium transporter activity is further regulated by phosphorylation in response to internal nitrogen status, with soil pH modulating the relative availability of ammonium versus nitrate and therefore the relative transcriptional demand on AMT versus NPF/NRT2 gene sets [15, 16]. The "steep, cheap, and deep" root ideotype - characterized by fewer, steeper axile roots with reduced metabolic cost - has been proposed as advantageous for nitrogen capture from deeper soil layers, though the genetic and transcriptional basis for coordinated expression of the multiple traits underlying this ideotype remains incompletely resolved [17]. The WFZP-TaSYD module discussed above provides one mechanistic bridge between root architectural regulation and nitrogen uptake efficiency, but comparable mechanistic studies linking specific root-architectural transcriptional regulators to nitrate/ammonium transporter expression remain scarce relative to the volume of purely descriptive DEG-cataloguing studies [14].

3.5 Phosphorus Transporter Transcriptional Regulation and Nitrogen-Phosphorus Interaction

Phosphorus (P) transporter regulation in wheat has been characterized largely independently of nitrogen-focused transcriptomics, yet the two nutrient-signalling pathways interact substantially at the transcriptional level. De novo transcriptome assembly of phosphate-starved wheat identified induction of the IPS1-mediated phosphate-starvation signalling cascade as a conserved adaptive mechanism shared with rice [18]. Genome-wide characterization of wheat PHT (phosphate transporter) genes demonstrated enhanced expression of PHT1-subfamily members in roots under phosphate starvation, consistent with their role in phosphate uptake, alongside distinct, tissue-specific PHT gene expression during grain filling and clear associations between TaPHT transcript abundance and phosphate accumulation in the aleurone layer [19, 20]. More recent work on Phosphorus Starvation Tolerance 1 (PSTOL1) homologues in wheat found differential, often genotype-dependent up- or down-regulation of individual TaPSTOL1 members under phosphorus deficiency versus sufficiency, indicating that - as with nitrate transporters - phosphorus transporter gene family regulation is not uniform but isoform- and genotype-specific [21]. ATP-binding cassette (ABC) transporter subfamily B genes, exemplified by TaABCB7, have similarly been implicated in the phosphate starvation response, broadening the transporter gene classes recognized as phosphorus-nitrogen-responsive beyond the canonical PHT1 family [22].

Direct transcriptomic evidence of nitrogen-phosphorus crosstalk in wheat specifically remains limited, but broader plant physiological literature and cereal NUE reviews note that nitrogen deficiency can alter phosphate homeostasis signalling, and vice versa, implying that nitrogen-responsive transcriptomic studies that do not simultaneously monitor phosphorus transporter transcript abundance may be missing a biologically relevant interaction effect. This is identified in Section 4 as a specific methodological gap in the current literature.

3.6 Potassium Transporter Transcriptional Response Under Variable Nutrient Regimes

Potassium (K+) deficiency responses have been comparatively profiled in low-potassium-susceptible and low-potassium-tolerant wheat genotypes, with the tolerant genotype exhibiting a greater number of up-regulated genes under potassium starvation, spanning metabolic process, cation-binding, transferase-activity, and ion-transporter gene ontology categories; genes associated with jasmonic acid signalling and defense response were also substantially induced under potassium deficiency [23]. While this potassium-focused transcriptomic literature is smaller in volume than the nitrogen-focused literature, it demonstrates a broadly analogous pattern - genotype-dependent transcriptional plasticity, tolerant genotypes mounting a larger and more coordinated transcriptional response than susceptible genotypes - that recurs across nitrogen, phosphorus, and potassium nutrient-deficiency transcriptomes in wheat, suggesting shared, convergent principles of nutrient-stress transcriptional architecture even where the specific transporter gene families differ.

3.7 Multi-Nutrient Regulatory Convergence and Emerging Regulatory Layers

Across the reviewed thematic areas, several convergent regulatory principles emerge. First, nutrient transporter gene

families in wheat are consistently large and functionally diversified as a consequence of hexaploidy, with homoeologous gene copies frequently showing divergent, sub- or neo-functionalized expression patterns rather than redundant co-regulation [1, 3]. Second, tissue specificity - particularly root-versus-shoot and vegetative-versus-reproductive divergence - is a near-universal feature of nutrient-responsive transcriptomes, cautioning against generalizing whole-plant conclusions from single-tissue sampling [4-6, 19]. Third, genotype-dependent variation in the magnitude and identity of differentially expressed genes is pervasive across nitrogen, phosphorus, and potassium studies alike, indicating that breeding-relevant transcriptional signatures are unlikely to be universal across the wheat genepool and must be validated across diverse germplasm [4, 5, 23]. Fourth, regulatory control operates across multiple molecular layers - transcription factors, chromatin remodelling, post-translational modification, and, increasingly, microRNA-mediated regulation - with cereal NUE reviews noting the growing recognition of microRNA-mRNA regulatory modules and their candidate-gene potential for genetic engineering, alongside CRISPR/Cas9-based editing of NRT1.1 and AMT1 orthologues already demonstrated to enhance nitrate and ammonium uptake efficiency in rice and, in select cases, wheat [16, 25].

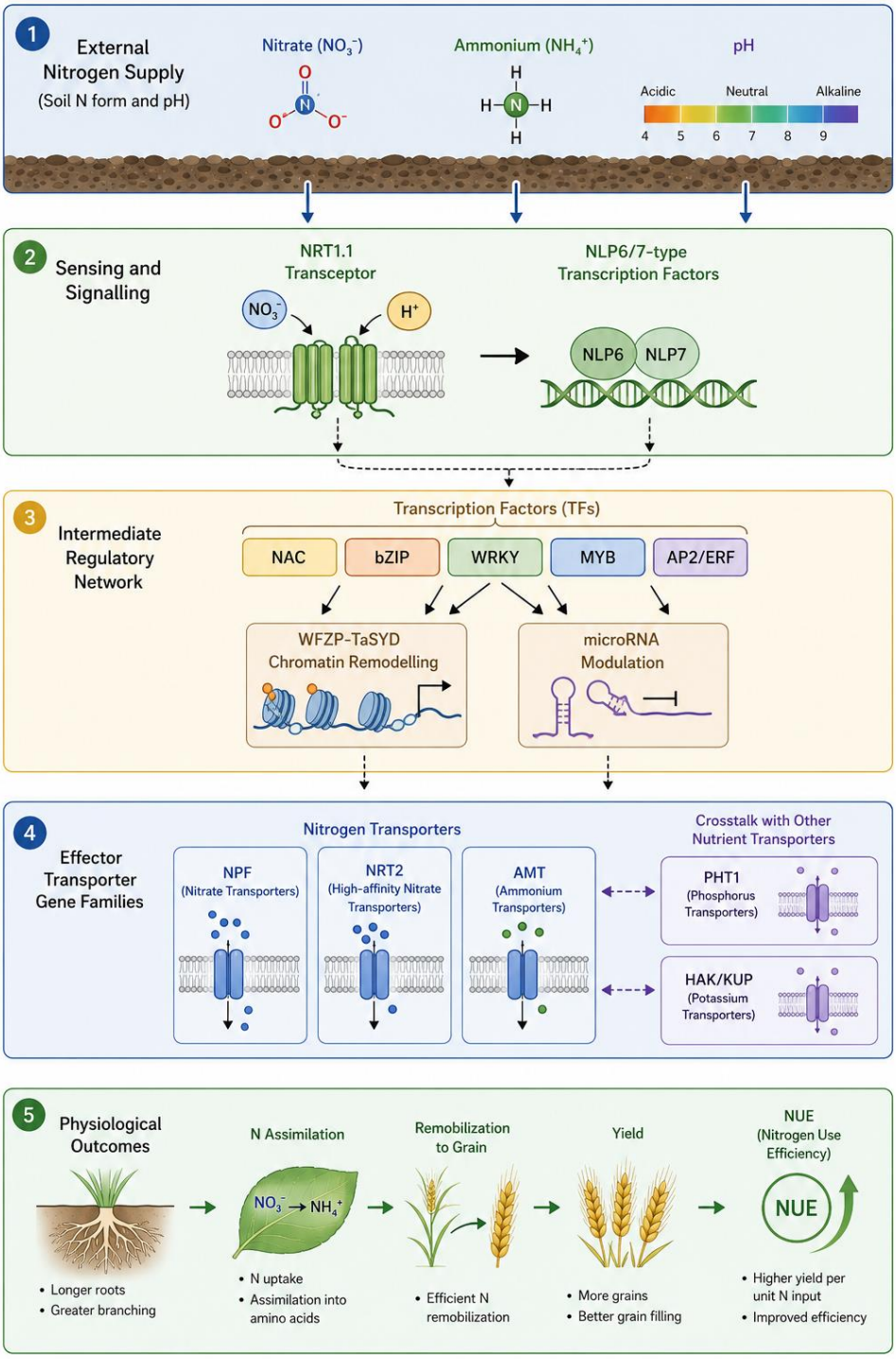

Fig 3: Conceptual framework of transcriptional regulation of nitrogen uptake pathways in wheat under variable nitrogen regimes

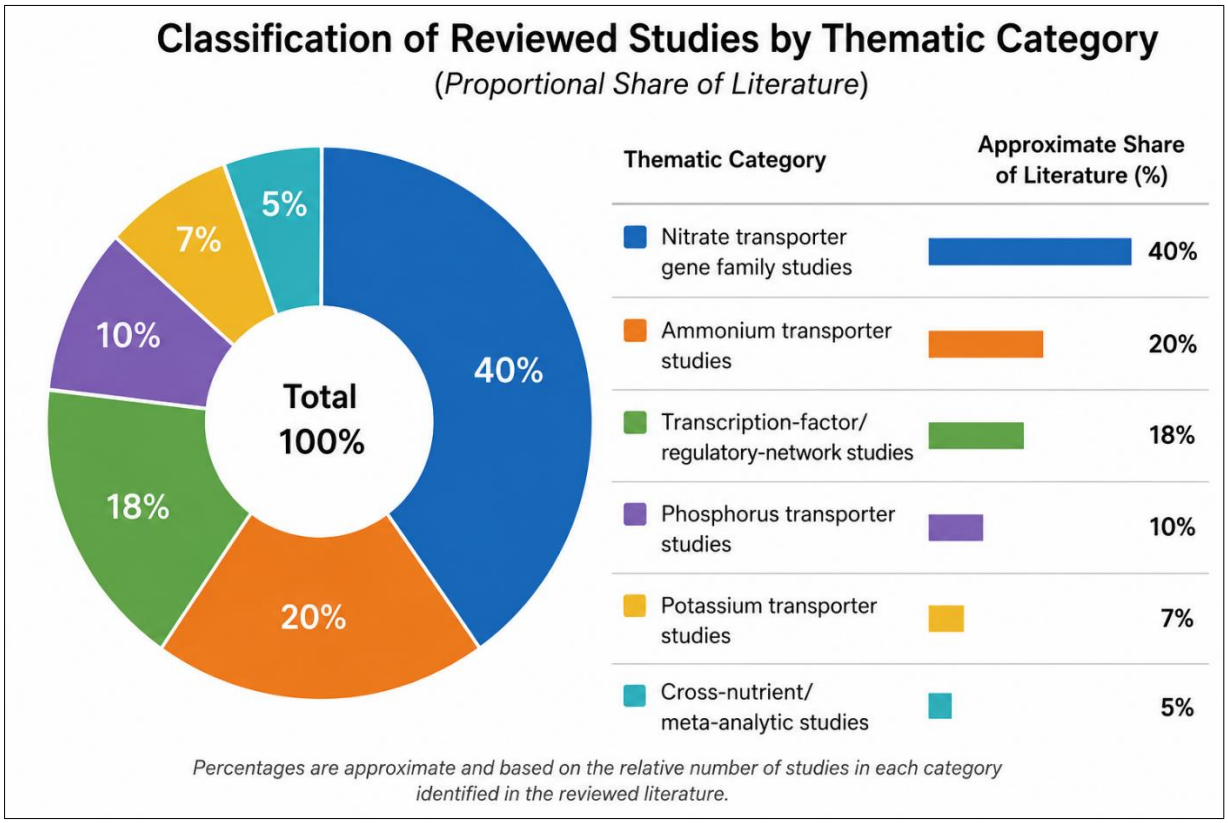

Fig 4: Thematic classification of the reviewed transcriptomic literature

4. Comparative Analysis of Previous Studies

Table 3 summarizes the principal transcriptomic studies reviewed, and Table 4 presents a comparative synthesis highlighting agreements, contradictions, and methodological limitations across the literature.

Table 3: Summary of key selected transcriptomic studies on nutrient uptake regulation in wheat

Author(s), Year	Study focus / location	Methodology	Major findings	Limitations
Wang *et al*., 2020	NPF gene family, China/UK (in silico + RNA-seq)	Genome-wide identification; RNA-seq across 6 tissues; qRT-PCR validation	331 NPF genes in 113 homoeologous groups; 44 genes with contrasting N/tissue expression identified	Expression atlas derived from limited cultivar panel; functional validation limited to select genes
Puccio *et al*., 2023	NPF/NRT2 family, durum wheat, Italy	Genome-wide characterization; RNA-seq, 9 tissues, 20 cultivars; WGCNA	211 NPF, 20 NRT2 genes; shared cis-regulatory elements; two NRT2 co-expression modules	Durum-specific findings may not fully generalize to hexaploid bread wheat
Kaur *et al*., 2022	Comparative NUE cultivars, India (PBW677 vs PBW703)	RNA-seq, root/shoot, control vs N stress	2,406 DEGs; efficient cultivar showed more abundant, coordinated DEGs including NRT1, TFs	Two-cultivar comparison; findings may not extend to broader germplasm
Sultana *et al*., 2020	Australian bread wheat cultivars (Mace, Spitfire, Volcani)	RNA-seq, leaf and grain, 0 and 10 days post-anthesis	12,344 DEGs; NRT1.1/1.2, nitrate reductase among most regulated genes	Reproductive-stage focus only; vegetative-stage dynamics not captured
Wang *et al*., 2024	Low-N stress gene network, wheat seedlings	RNA-seq; WGCNA across 47 datasets	8,468 DEGs; opposite root/shoot regulation; 5 candidate hub TFs identified	Hub TFs not yet functionally validated in planta
Du *et al*., 2025	WFZP-TaSYD module, China	Mutant phenotyping; ChIP; transcriptomics	WFZP-TaSYD promotes root development and nitrate-uptake gene expression via chromatin remodelling	Mechanism demonstrated primarily in mutant/model lines; field validation limited
Shukla *et al*., 2016	Wheat PHT gene family and grain P allocation	RNA-seq/qRT-PCR across tissues and grain-filling stages	12 new PHT genes; PHT1 induced under Pi starvation; tissue-specific grain expression	Focus on P only; no simultaneous N-regime comparison
Guo/authors, 2015	K+-deficiency transcriptomes, tolerant vs susceptible genotype	RNA-seq, comparative genotypes under K+ starvation	Tolerant genotype showed larger, coordinated up-regulation of ion-transporter genes	Two-genotype design; jasmonic acid pathway role not functionally confirmed

Table 4: Comparative analysis: agreements, contradictions, and evidentiary strength across reviewed themes

Theme	Points of agreement across studies	Points of contradiction / heterogeneity	Relative strength of evidence
Nitrate transporter (NPF/NRT2) regulation	Tissue-specific and N-responsive expression; genotype-dependent DEG abundance; NUE-efficient genotypes show more coordinated regulation	Direction of NRT1.1 regulation (up vs down under stress) varies by study/stage; N-dose thresholds inconsistent	Strong (multiple independent genome-wide and comparative RNA-seq studies)
Ammonium transporter (AMT) regulation	High-affinity AMT1 isoforms induced under N deprivation; post-translational feedback conserved with Arabidopsis	Limited number of wheat-specific studies; nitrate-ammonium interactive regulation rarely tested jointly	Moderate (fewer independent studies than NPF/NRT2)
Transcription factor networks	NLP family upstream sensors; NAC/bZIP/AP2-ERF intermediate regulators; some factors (TabZIP60) act as negative NUE regulators	Identity of dominant hub TFs differs across cultivars/tissues; some regulatory directions appear cultivar-specific	Moderate-strong (functional validation available for several factors)
Phosphorus transporter crosstalk	PHT1 induction under Pi starvation; tissue-specific grain PHT expression	Rarely studied concurrently with N regimes; crosstalk mechanisms largely inferred rather than directly tested	Weak-moderate (indirect evidence predominant)
Potassium transporter response	Genotype-dependent transcriptional magnitude; tolerant genotypes show larger coordinated response	Very few wheat-specific K+ transcriptomic studies identified	Weak (limited independent replication)

5. Research Gaps and Emerging Trends

Several unresolved issues limit the translational value of current transcriptomic evidence. First, inconsistent experimental design - varying nitrogen-dose definitions of "low" versus "sufficient" N, differing developmental stages sampled, and non-standardized tissue collection protocols - substantially constrains meta-analytic synthesis across studies [4-6]. Second, most transcriptomic studies remain descriptive, cataloguing DEGs and enriched gene ontology terms without functional validation (for example, via CRISPR knockout, RNAi, or overexpression) of candidate hub regulators, leaving causal relationships between specific transcription factors and NUE outcomes only partially established [6, 14]. Third, field-based validation of greenhouse- or hydroponics-derived transcriptomic findings is rare, raising questions about the applicability of controlled-environment gene-expression signatures to variable, real-world soil nitrogen dynamics [13]. Fourth, integration across nutrient classes - simultaneous transcriptomic profiling of nitrogen, phosphorus, and potassium transporter families under combined or interacting nutrient regimes - remains uncommon despite physiological evidence of crosstalk, representing a significant methodological gap. Fifth, reference genome annotation versions differ across studies conducted before and after major wheat genome assembly updates, complicating gene-identifier cross-referencing and reproducibility [1, 3]. Table 5 summarizes these identified research gaps, and Table 6 outlines emerging technologies and trends positioned to address them.

Table 5: Research gaps identified in the reviewed literature

Gap Category	Description	Consequence
Experimental standardization	Inconsistent N-dose thresholds, growth stages, and tissue sampling protocols	Limits cross-study comparability and meta-analytic synthesis
Functional validation	Predominance of descriptive DEG/GO-enrichment studies over CRISPR/RNAi validation	Causality between candidate regulators and NUE outcomes remains unconfirmed
Field translation	Most transcriptomic data derived from hydroponic/greenhouse conditions	Uncertain applicability to variable field soil-nitrogen dynamics
Multi-nutrient integration	Few studies jointly profile N, P, and K transporter transcription	Crosstalk mechanisms remain inferential rather than directly demonstrated
Genome annotation consistency	Studies span multiple wheat reference genome/annotation versions	Complicates gene identifier mapping and reproducibility across studies
Ammonium-nitrate joint regulation	Few studies examine combined nitrate/ammonium transcriptional interaction in wheat specifically	Incomplete understanding of mixed-N-source field conditions

Table 6: Emerging technologies and trends relevant to future transcriptomic research on wheat nutrient uptake

Emerging trend	Description	Anticipated contribution
CRISPR/Cas9 gene editing	Targeted editing of NRT1.1, AMT1, and related transporter orthologues, already applied in rice and increasingly in wheat	Direct functional validation and precision breeding for NUE
microRNA-mediated regulation	Characterization of miRNA-mRNA modules (e.g., miR166-Dof interactions) regulating nutrient transporter expression	Additional regulatory layer for candidate-gene discovery
Single-cell and spatial transcriptomics	Cell-type-resolved expression profiling of root tissue under variable N supply	Finer-resolution mapping of transporter expression beyond bulk tissue averages
Multi-omic integration	Combining transcriptomics with proteomics, metabolomics, and ionomics	Systems-level understanding of nutrient uptake regulation
Genomic selection and GWAS	Marker-trait association studies under combined stress (e.g., heat + low N)	Identification of pleiotropic loci for breeding programmes
Machine-learning network inference	Computational reconstruction of gene regulatory networks from large transcriptome compendia (e.g., WGCNA-based hub identification)	Prioritization of candidate regulatory hub genes for functional testing

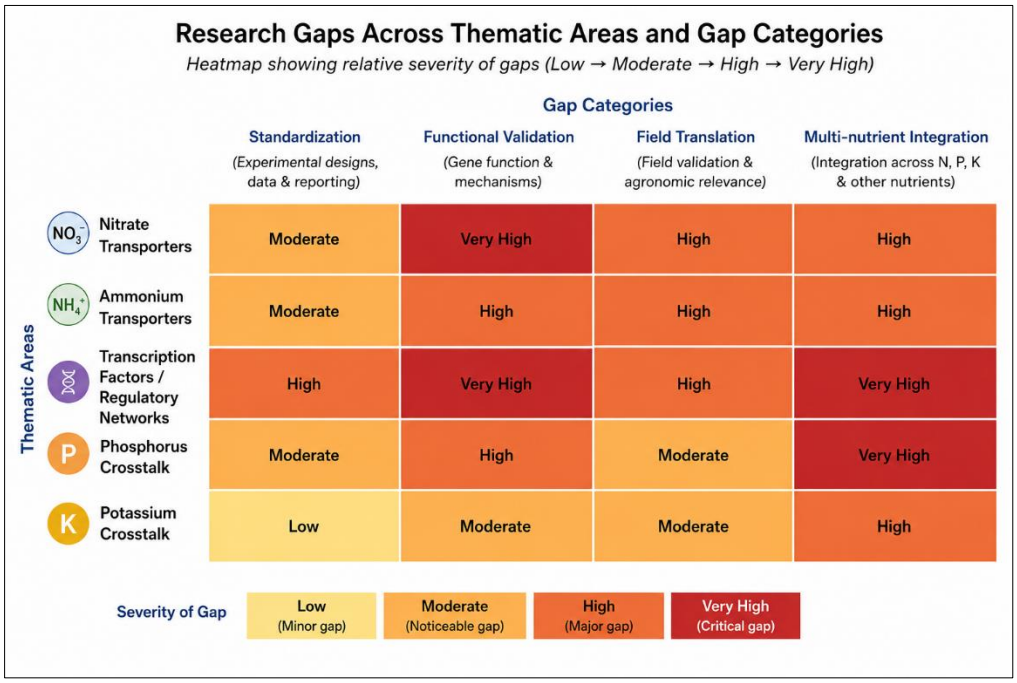

Fig 5: Research gap mapping across thematic areas of wheat nitrogen transcriptomics

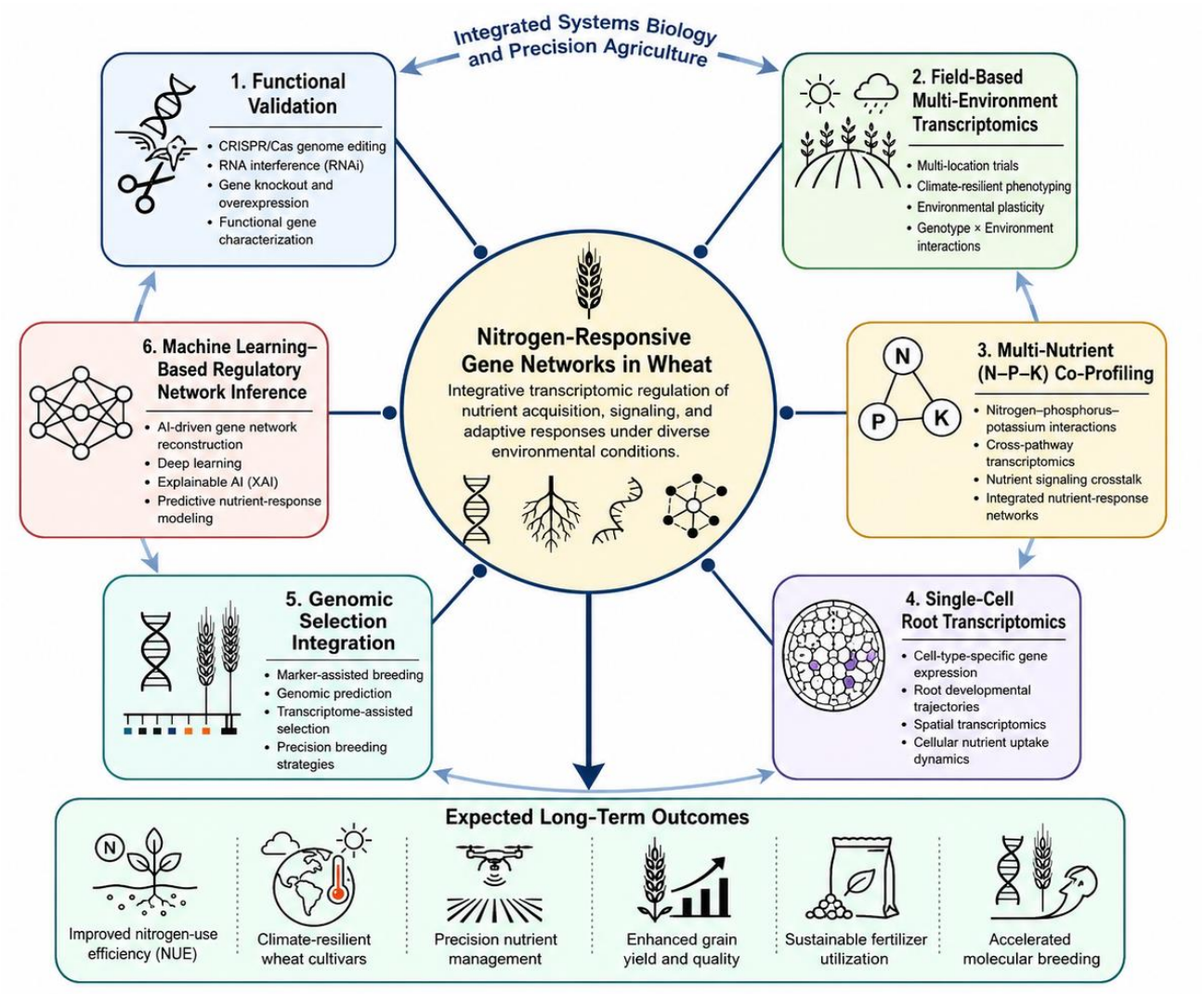

Fig 6: Future research directions for transcriptomic regulation of nutrient uptake in wheat

6. Discussion

The synthesized evidence indicates that nitrogen uptake pathway regulation in wheat is governed by a hierarchical, multi-layered transcriptional architecture in which upstream nitrate-sensing transcription factors (principally NLP family members), intermediate genotype- and tissue-specific

regulators (NAC, bZIP, AP2/ERF, MYB, WRKY), and chromatin-remodelling complexes converge to control the expression of large, functionally diversified nitrate (NPF, NRT2) and ammonium (AMT) transporter gene families [1, 3, 6, 9, 14]. This layered model is broadly consistent across independent studies conducted on different wheat genetic backgrounds (Indian, Australian, Chinese, and Italian durum germplasm), which lends confidence to its generalizability as a conceptual framework, even though the identity of the dominant hub regulators and the precise direction of individual transporter gene regulation vary across studies [4, 5, 6].

A recurring and biologically important pattern is that nitrogen-efficient wheat genotypes do not simply express nitrate transporter genes more strongly; rather, they exhibit a larger, more coordinated transcriptional response encompassing nitrogen assimilation, carbon metabolism, and stress-signalling pathways simultaneously [4, 23]. This observation - replicated independently in the potassium-deficiency literature, where tolerant genotypes likewise mount a broader coordinated transcriptional response than susceptible genotypes - suggests that NUE (and, by extension, general nutrient-use efficiency) may be underpinned less by a single rate-limiting transporter gene and more by the degree of transcriptional network coordination across metabolic and transport pathways [4, 23]. This interpretation has practical implications for breeding: selection strategies targeting single candidate transporter genes may yield more limited NUE gains than approaches that select for coordinated regulatory network architecture, for example via genomic selection incorporating multiple transcription-factor loci simultaneously [16].

Conflicting evidence in the literature - particularly regarding the direction of NRT1.1 regulation under nitrogen stress - appears attributable primarily to methodological heterogeneity (developmental stage, tissue, N-dose threshold) rather than genuine biological contradiction, though this cannot be confirmed without harmonized, multi-site replication studies. The demonstrated negative regulatory role of TabZIP60 further complicates any simplistic "more nitrogen-responsive gene expression equals higher NUE" narrative, since TabZIP60 down-regulation, not up-regulation, of the associated NADH-GOGAT pathway improved yield [13]. This finding is a useful corrective within the field: transcriptomic DEG catalogues alone cannot indicate whether a given regulatory relationship is agronomically beneficial or detrimental, and functional validation - as performed for TabZIP60, TaNAC2, and WFZP-TaSYD - remains indispensable before candidate genes are prioritized for breeding or gene-editing pipelines [8, 12, 14].

The comparatively underdeveloped literature on nitrogen-phosphorus-potassium transcriptional crosstalk in wheat, relative to the extensive nitrate-transporter literature, represents both a limitation of the current evidence base and an opportunity for the field. Given that global fertilizer management is typically not nitrogen-only, but instead involves combined N-P-K applications whose relative balance interacts with soil pH and cation/anion uptake competition, transcriptomic studies that profile only nitrogen-responsive genes may be capturing a partial, potentially confounded picture of nutrient-transporter regulation in realistic agronomic settings [15, 16, 18-20, 23]. Multi-nutrient co-profiling, ideally under field or near-field conditions, would meaningfully strengthen the evidentiary basis for nitrogen-focused breeding recommendations derived from transcriptomic studies.

7. Conclusion

This work has summarized transcriptomic data regarding the regulation of nutrient uptake mechanisms in wheat under varying nitrogen conditions. It has covered the nitrate (NPF, NRT2) and ammonium (AMT) transporter gene family members, the genes coding their transcription factors and chromatin-remodeling regulators, and their links with genes involved in phosphorus and potassium transport. The main findings of literatures, however, suggest the existence of a hierarchical model of nitrogen uptake regulation that is tissue- and variety-specific. The nitrogen-efficient varieties are less differentiated from others by one of the transporter gene expressions, but by the overall transcription network of the transporter, assimilation, and stress response pathways. Nevertheless, the evidence base remains very limited in terms of methodological approach, predominance of descriptive findings over functionally validated ones, lack of replication in field studies, and rarity of simultaneous study of several nutrients.

According to this review, researchers should place emphasis on the need to standardize experimental protocols (the definitions for nitrogen-dose should be standardized, including developmental staging as well as tissue sampling), improving the validation of possible regulatory genes through different approaches like WGCNA, along with multi-nutrient (N-P-K) transcriptome co-sampling at field conditions. Furthermore, practitioners and policy-makers working in the field of wheat breeding and fertilizer management should interpret the results with care, highlighting the necessity to favor the usage of modern breeding techniques involving the regulatory networks and not just focusing on genes responsible for the transporter alone.

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