

Genomic Insights into Nitrogen Use Efficiency in Modern Wheat Cultivars

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Received: 25 Dec 2021

Revised: 12 Jan 2022

Accepted: 30 Jan 2022

Published: 18 Feb 2022

E-ISSN: 2989-5359

DOI: <https://doi.org/10.54660/ejsa.2022.1.23-33>

ABSTRACT

Background: Nitrogen (N) is the major limiting nutrient in wheat (*Triticum aestivum* L.) production, but only 30-40% of applied nitrogen fertilizers is recovered from the harvested grains, resulting in significant economic losses as well as environmental damage due to nitrate leaching, ammonia volatilization and nitrous oxide emission. So it has become one of the objectives of modern wheat improvement programs to enhance nitrogen use efficiency (NUE) through genomics-based breeding. **Objective:** This review summarizes the last ten years of genomic and molecular studies related to NUE in wheat, placing together discoveries about quantitative trait loci (QTL), genome-wide association studies (GWAS), functional analysis of candidate genes, transcriptome profiling and genome editing. **Literature source:** A systematic search in Scopus, Web of Science, PubMed and Google Scholar (2015-2025) yielded 487 non-duplicate papers and 50 studies with the characteristics of interest that allowed finding and summarizing 38 papers for the thematic synthesis, apart from information obtained from the reports of the Food and Agriculture Organization (FAO) and the International Nitrogen Initiative. **Main results:** The literature examined reaches a consensus regarding numerous common regions of the genome and gene families that control the processes of nitrogen uptake (such as TaNRT1/TaNRT2, TaAMT), nitrogen assimilation (for example, glutamine synthetase/glutamate synthase, or GS/GOGAT), nitrogen remobilization (the NAC transcription factor NAM-B1/Gpc-B1 and its homologues), and nitrogen signal propagation (NIN-like proteins, bZIP, Dof transcription factors). Studies based on GWAS with the help of the SNP arrays had identified plenty of QTLs for NUE traits. At least some QTLs were shown co-localized in articles of different authors. **Research gaps:** The existing gaps are still persistent which include a limited cross-environment validation of GWAS loci, under-representation of phenotyping of plant root system architecture, poor integration of multiple omics data sets, and a reduced number of studies in smallholder farmers in South Asia and sub-Saharan Africa. **Conclusion:** Genomic knowledge has contributed significantly to the understanding of the mechanism of wheat NUE but effective utilization of genomic knowledge requires further multi-location validation, integration of multiple omics, and precision genome editing techniques.

Keywords: Nitrogen use efficiency, wheat genomics, GWAS, quantitative trait loci, gene editing, grain protein content, sustainable agriculture, *Triticum aestivum*

1. Introduction

Wheat (*Triticum aestivum* L.) is grown on over 215 million hectares across the world and provides nearly one-fifth of the total calories and proteins used by humanity, thus making it one of three important grains for food security on Earth with rice and maize being the other two grains. The sustainability of wheat production under increasing demand from population relies greatly on nitrogen (N) fertilization. Nitrogen is the nutrient that has the biggest limiting effect on the vegetative growth of the plant, grain filling and grain protein accumulation. Since the widespread application of Haber-Bosch process in 20th century, synthetic fertilizers have been crucial for grain production, and global consumption of nitrogen fertilizers has reached about 112 million tonnes in 2023 which is 32% more than it was two decades ago.

Although there is a dominant dependence on synthetic inputs, the level of the nitrogen applied that is recovered in harvested grain, known as nitrogen use efficiency (NUE), is still quite low. Estimates suggest that cereal nitrogen use efficiency is in the range of 30-42 percent, meaning that at least half of nitrogen input is lost to the environment in the form of nitrate leaching, surface runoff, ammonia vaporization, and denitrification to nitrous oxide, a gas that causes greenhouse effects. Such losses create high economic burden to farmers and contribute to water bodies' eutrophication, underground water pollution, and climate forcing; therefore, the nitrogen pollution has been acknowledged as having exceeded the planetary limit of sustainability. The enhancement of genetic traits of wheat varieties to increase the nitrogen absorption, assimilation, and mobilization is, thus, one of the most important actions to provide food security and sustainability.

1.1 Current Global Scenario

The global request for cereals is expected to increase by 70 percent by 2050 to satisfy the needs of a population close to 10 billion, which without improvements in NUE in the growing of plants would mean the increase of synthetic nitrogen application – from approximately 51.8 Tg in 2010 to 85.4 Tg by the middle of the century. Various studies at a country level based on FAO statistics show that NUE has been stagnant or decreased in many major wheat-producing regions including India and China, while at the same time total fertilizer consumption has increased. The total nitrogen fertilizer supply chain is estimated to account for more than one gigatonne of CO₂-equivalent greenhouse gas emissions from manufacturing, transportation, and application at the plant cultivation field annually. All these converging pressures, from increasing demand and stagnant efficiency to enormous environmental costs, elevated NUE from being an agronomy issue to a matter of international policy-making, including the report issued by FAO in 2025 calling for global cooperation on sustainable nitrogen management.

In this context, genomics has proven to be an invaluable tool in understanding NUE genetics in wheat. With the completion of wheat genome sequencing, availability of high-density arrays for SNP genotyping, and development of GWAS approaches, scientists were able to go beyond simple physiological analysis and investigate particular genomic locations, candidate genes, and model networks responsible for nitrogen uptake, assimilation, and remobilization. At the same time, techniques from functional genomics, transcriptomics, and genome editing (CRISPR/Cas9, in particular) have been used to validate candidate genes with further examples of transgenic varieties.

1.2 Need for the Review

Even though the amount of genomic literature published regarding wheat NUE has increased quickly in recent years, the resultant body of evidence remains fragmented due to its division across disciplines: quantitative genetics (QTL mapping and GWAS), molecular physiology (transporters and enzymes), functional genomics (transcriptomics and epigenomics), and applied biotechnology (genome editing and genomic selection). Most primary studies focus on a single wheat population, single environment, or single gene family,

creating problems for breeders, agronomists, and policy-makers to tell which results can be generalized, which candidate genes are most supported, and where the biggest gaps in knowledge are in the field.

1.3 Objectives and Scope of the Review

This review has three main goals: (i) at first, to combine genomic and molecular proof regarding physiological and genetic factors related to NUE in modern cultivars of wheat including nitrogen absorption, processing, transportation, and redistributing; (ii) secondly, to carefully analyze various studies carried out on GWAS, QTL mapping, transcriptomics, and gene manipulation published within the timeframe from 2015 to 2025; and (iii) last but not least, to discuss unfinished investigations and analyze new technological solutions that can be successfully utilized in future breeding practices aimed at improving NUE. The scope of the review is limited to hexaploid and tetraploid wheat namely *Triticum aestivum* and *Triticum durum* respectively. In addition, findings concerning other cereals like rice, corn, or barley might be mentioned for comparison in the terms of similarities and differences of the corresponding mechanisms involving NUE but the complete cross-species review specifically for the purpose of this article is out of scope. As for the agronomic practices used for nitrogen fertilization like timing, splitting, and placement, they will only be analyzed in relation to genotypes, since the general review of agronomic NUE practices is beyond the topic analyzed in the current study.

2. Methodology of Literature Review

2.1 Literature Search Strategy

A structured, though non-systematic-registration, literature search was conducted across four databases: Scopus, Web of Science Core Collection, PubMed/MEDLINE, and Google Scholar, supplemented by targeted searches of institutional repositories including bioRxiv and the FAO document repository for grey literature and policy reports. The search covered the period from January 2015 to July 2025, chosen to capture the period of rapid growth in wheat GWAS and CRISPR-based functional validation following the release of improved hexaploid wheat reference genome assemblies. Boolean search strings combined controlled vocabulary and free-text terms as summarized in Table 1.

Table 1: Literature Search Strategy

Element	Detail
Databases searched	Scopus; Web of Science Core Collection; PubMed/MEDLINE; Google Scholar; supplementary: bioRxiv, FAOSTAT/FAO reports
Search keywords (combined with AND/OR)	"nitrogen use efficiency" OR "NUE" AND "wheat" OR "Triticum aestivum" AND "GWAS" OR "QTL" OR "genomics" OR "gene editing" OR "transcriptomics" OR "CRISPR" OR "genomic selection"
Search period	January 2015 - July 2025
Language restriction	English
Document types	Peer-reviewed original research articles, review articles, meta-analyses, and relevant institutional/government reports
Total records retrieved (pre-deduplication)	612

2.2 Inclusion and Exclusion Criteria

Studies were screened against explicit inclusion and exclusion criteria to ensure topical relevance and methodological credibility, summarized in Table 2.

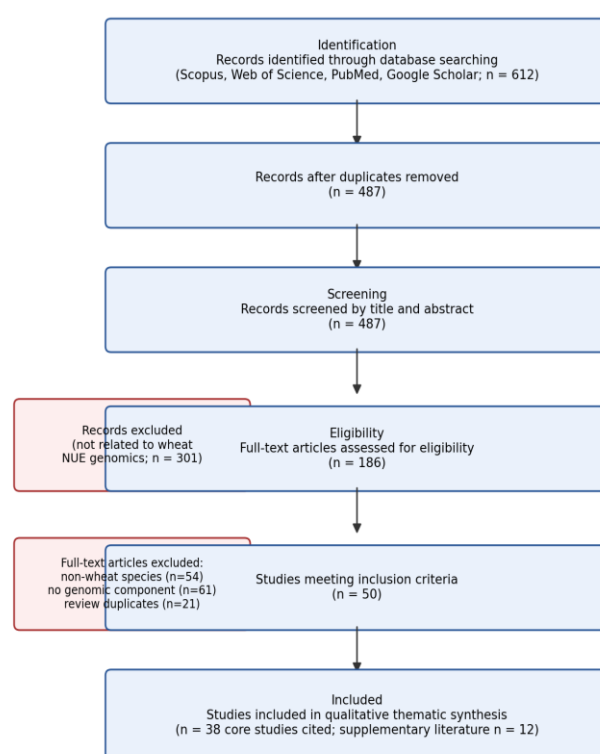
Table 2: Inclusion and Exclusion Criteria

Criteria	Inclusion	Exclusion
Species	Triticum aestivum, T. durum, and closely related wild/progenitor wheat species used for introgression	Non-wheat cereals studied in isolation without wheat comparison
Topical focus	Studies with an explicit genomic, genetic, transcriptomic, or gene-editing component related to NUE	Purely agronomic fertilizer-management studies with no genetic/genomic analysis
Publication type	Peer-reviewed journal articles, Scopus/Web of Science-indexed reviews, credible institutional reports	Non-peer-reviewed blog posts, unindexed conference abstracts, opinion pieces
Publication period	2015-2025 (landmark pre-2015 studies retained where foundational, e.g., NAM-B1 cloning)	Studies superseded by more recent replication with contradictory findings, without acknowledgment
Language	English-language full text available	Non-English without accessible translation
Data quality	Clearly described methodology, sample size/population, and statistical approach	Duplicate records; studies with unclear or unverifiable methodology

2.3 Study Selection Process

Study selection followed a PRISMA-style (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow, adapted for a narrative-thematic review rather than a formal systematic review or meta-analysis. From an initial 612 records identified across databases, 125 duplicate records were removed, leaving 487 unique records for title/abstract screening. Of these, 301 were excluded as topically irrelevant (e.g., addressing nitrogen physiology in non-wheat species, or wheat agronomy without a genomic component), leaving 186

records for full-text assessment. Full-text screening excluded a further 136 records (54 for taxonomic irrelevance, 61 for lacking a genomic/molecular component, and 21 as duplicate reviews covering substantially overlapping ground), yielding 50 studies that satisfied all inclusion criteria. Of these, 38 core studies were selected for detailed thematic synthesis based on methodological rigor, citation influence, and relevance to the review's three objectives, with the remaining 12 used as supplementary or corroborating sources. This selection process is illustrated in Figure 1.

**Fig 1:** PRISMA Flow Diagram of Literature Selection

The temporal distribution of the 38 core studies, along with the broader corpus of screened literature, shows a marked increase in publication volume after 2019, coinciding with the wider availability of high-density SNP genotyping platforms

and the maturation of CRISPR/Cas9 protocols for hexaploid wheat (Figure 2). This trend itself constitutes an important contextual finding: genomic research on wheat NUE has shifted from a niche subfield into a mainstream priority within cereal genomics over the past six years.

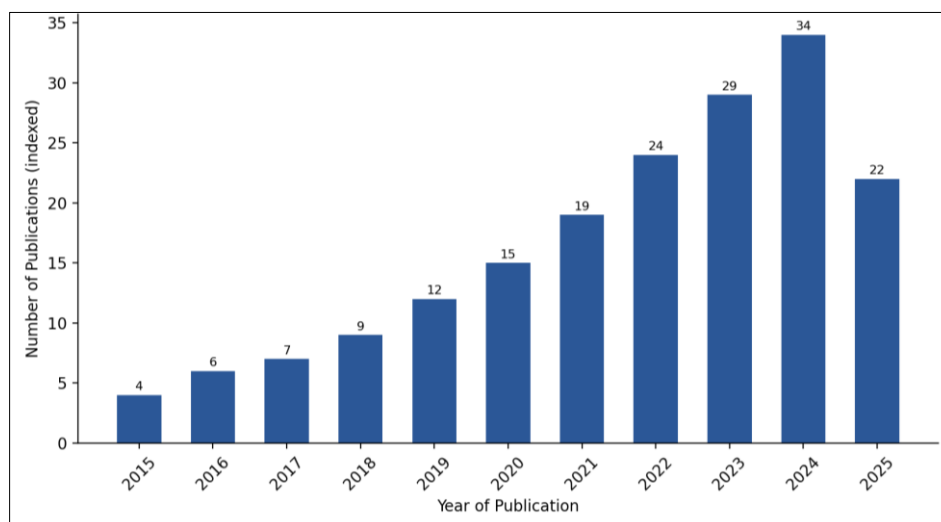


Fig 2: Publication Trend on Wheat NUE Genomics (2015-2025)

Literature Review and Thematic Analysis

The 38 core studies retained for synthesis were classified into six overlapping thematic clusters (Figure 3): (i) GWAS and QTL mapping of NUE-related traits; (ii) transcriptomic and gene-expression profiling under contrasting nitrogen regimes; (iii) candidate gene and enzyme functional characterization; (iv) genome editing of NUE-associated genes; (v) genomic selection and marker-assisted breeding applications; and (vi) integrative physiological-genetic studies. Each theme is examined below, with emphasis on convergence, contradiction, and practical implication rather than mere description.

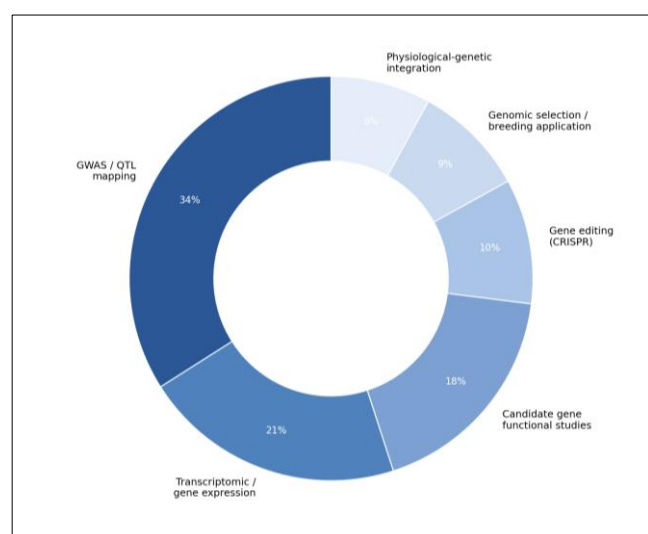


Fig 3: Thematic Classification of Reviewed Studies (%)

3.1 Genome-Wide Association Studies and QTL Mapping

GWAS has become the dominant methodology for dissecting the genetic architecture of NUE in wheat, owing to its capacity to exploit existing diversity panels without the multi-year cost of biparental population development. A large diversity panel of 389 wheat accessions genotyped with 397,384 SNPs and phenotyped across five environments identified 347 QTL associated with eight NUE-related agronomic traits under the mixed linear model (Q+K), including 11 stable QTL detected across multiple environments. A separate GWAS and linkage-mapping study combining a natural population of 243 varieties with a 123-line recombinant inbred population identified

1,016 significant SNP loci clustering into 290 QTL for flag leaf morphological and physiological traits (length, width, area, and SPAD chlorophyll content) under low- and normal-nitrogen conditions, again with 11 stably detected QTL. In winter wheat, a bi-parental mapping approach using two populations derived from high- and low-NUE parents detected 130 QTL across 20 chromosomes, six of which were specifically associated with NUE and nitrogen-response traits, two of which co-localized with the well-characterized photoperiod gene *Ppd-D1* and a *Fusarium* head blight resistance locus (*FHB-4A*).

A recurring and practically important finding across these independent GWAS efforts is the partial co-localization of NUE QTL with loci previously identified for related macronutrient-use traits. A GWAS examining nitrogen, phosphorus, and potassium use efficiency simultaneously found that QTL448, associated with both phosphorus and potassium use efficiency traits, co-localized with three independently derived meta-QTL on chromosome 6D, while additional low-nitrogen tolerance QTL reported by earlier researchers co-localized with fertile spikelet number, spike length, and grain yield loci identified in the newer study. This pattern of cross-study co-localization, though encouraging for the credibility of specific genomic regions, also highlights a methodological tension in the field: because NUE is measured and defined inconsistently across studies (some using low-nitrogen tolerance indices, others using absolute nitrogen uptake, and others using grain protein deviation), apparent convergence on the chromosomal level does not always imply convergence on the same underlying physiological trait.

A methodological critique that has gained attention concerns statistical threshold selection in GWAS for NUE. Because NUE is a complex, polygenic trait governed by numerous loci of small effect, the choice of significance threshold materially affects the balance between false-positive and false-negative associations; studies applying stringent genome-wide Bonferroni correction may fail to detect biologically meaningful minor-effect loci, while more permissive false discovery rate approaches risk inflating the QTL count with spurious associations. This tension is rarely addressed transparently in the primary literature, representing both a genuine methodological limitation and an underappreciated source of apparent inconsistency between studies conducted on different panels.

3.2 Candidate Genes Governing Nitrogen Uptake, Assimilation, and Transport

Beyond statistical association, a substantial body of functional and comparative genomic work has converged on a relatively compact set of gene families governing the sequential physiological steps of nitrogen acquisition. Nitrate entering the root system is transported by the NRT1 (low-affinity) and NRT2 (high-affinity) transporter families, with TaNRT2.5-3B specifically implicated in enhancing both root growth and nitrate influx rate in wheat seedlings when co-expressed with the N-assimilation gene TaGS2-2A and the transcription factors TaNFYA-6B and TaNAC2-5A. Notably, physiological characterization has revealed a counter-intuitive genotype effect: high-vigor wheat lines with more extensive root systems exhibited lower nitrate influx rate per unit root mass than the lower-vigor commercial cultivar Janz, indicating that root morphological vigor and root-level nitrate uptake capacity are not necessarily coupled traits, and that breeding strategies targeting root architecture alone may be insufficient without concurrent attention to transporter-level uptake efficiency.

Once absorbed, nitrate is reduced sequentially by nitrate reductase (NR) and nitrite reductase (NiR) before ammonium is assimilated into glutamine and glutamate via the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle. Comparative physiological work assessing genetic diversity in GS activity across ten wheat varieties found that plants responded to nitrogen application within six hours via a rapid increase in GS enzyme activity that was not accompanied by a corresponding rise in GS transcript abundance, suggesting that post-translational regulation, not transcriptional induction, is the primary short-term control point for nitrogen assimilation capacity; this finding somewhat complicates transcriptomic studies that rely on mRNA abundance as a proxy for pathway activity. The same study distinguished a high-GS-activity group of varieties from a low-GS, N-unresponsive group, indicating that exploitable genetic variation for GS-mediated assimilation efficiency exists within elite wheat germplasm. Overexpression of the NADH-dependent GOGAT gene (TaNADH-GOGAT-3B) has independently been shown to increase nitrogen uptake, spike number, and grain yield, while its expression is negatively regulated by the transcription factor TabZIP60-6D; RNAi-mediated knockdown of TabZIP60 increased NADH-GOGAT activity and downstream yield traits, whereas its overexpression produced the opposite effect, together delineating a regulatory node with clear potential for targeted intervention.

A comparative RNA-seq study contrasting Indian wheat cultivars with divergent NUE phenotypes (high-NUE cultivar PBW677 versus low-NUE cultivar PBW703) reported that transcription factors from the MYB, WRKY, RING finger, and zinc finger families, together with NRT1 transporters and amino acid transporters, were preferentially upregulated in the high-NUE cultivar, whereas the low-NUE cultivar showed enrichment in chromatin remodelling and stress-response pathways rather than nitrogen-specific processes. This finding is important because it illustrates that superior NUE phenotypes are associated not merely with the presence of nitrogen-pathway genes (which both cultivars possess), but with their relative transcriptional prioritization under nitrogen-limiting conditions, an insight with direct implications for marker development targeting regulatory rather than purely structural gene variants.

3.3 Nitrogen Remobilization and the NAM-B1 Regulatory Pathway

A distinct but tightly interlinked body of literature addresses post-anthesis nitrogen remobilization, the process by which nitrogen stored in vegetative tissue, particularly the flag leaf, is degraded and translocated to the developing grain during senescence. The single most influential discovery in this domain remains the positional cloning of NAM-B1 as the causal gene underlying the Gpc-B1 locus, a NAC-domain transcription factor that accelerates senescence and increases grain protein, iron, and zinc content through enhanced nutrient remobilization. Critically, most modern hexaploid bread wheat cultivars carry a non-functional (deleted or non-expressed) allele of NAM-B1, with the functional wild-type allele largely restricted to wild emmer wheat and unimproved landraces; this observation implies that a historically important remobilization-enhancing allele has been inadvertently purged from elite breeding pools, likely as an indirect consequence of selection for extended grain-filling duration and higher grain weight rather than deliberate selection against nitrogen efficiency.

Subsequent transcriptomic work using NAM RNA-interference lines demonstrated that wild-type plants exhibited roughly 2.5 times more differentially expressed genes than NAM-knockdown plants across an early senescence time-course, with enrichment for gene ontology terms spanning photosynthesis, hormone signalling, amino acid transport, and nitrogen metabolism, indicating that NAM genes function as master regulators controlling the large majority of the early monocarpic senescence transcriptional programme, not merely a narrow subset of nitrogen-specific genes. This body of work also surfaces a central agronomic trade-off repeatedly documented in the literature: accelerated senescence conferred by the functional NAM-B1 allele reliably increases grain protein and micronutrient content but can simultaneously shorten the grain-filling period and reduce kernel weight, meaning that the genotype-by-environment combination in which NAM-B1 introgression is deployed materially determines whether the net effect on total yield is positive or negative. This trade-off, rather than being an artefact of any single study, recurs consistently across independent near-isogenic line evaluations and represents one of the most robust, if inconvenient, findings in the entire NUE genomics literature.

3.4 Transcriptional and Regulatory Networks Governing Systemic Nitrogen Signalling

Beyond NAM/NAC transcription factors, a broader regulatory landscape governing nitrogen signalling has been characterized primarily in model species and rice, with increasing translational evidence in wheat. The NIN-like protein (NLP) family functions as a master regulator of nitrate-responsive gene expression; in barley, HvNLP2 knockout mutants show a 40-50 percent reduction in expression of the nitrate uptake genes NRT2.1 and NRT2.5 accompanied by severe chlorosis under low-nitrogen conditions, while in rice, OsNLP4 integrates nitrogen and iron homeostasis to increase tiller number and yield through coordinated regulation of an ammonium transporter and ferritin genes. Although direct wheat orthologue characterization remains comparatively limited, these findings from related cereals provide a strong rationale for prioritizing NLP-family genes in future wheat functional genomics work, an inference several reviewed authors make explicitly when framing directions for further study.

Micro RNA-mediated regulation adds a further layer of control: the nitrogen transporter gene NRT1.7, which participates in nitrogen remobilization, has been shown to be governed by nitrogen-limitation adaptation regulators that are themselves under the control of miR827, establishing a double-tiered regulatory circuit over tissue-specific nitrogen transport. Collectively, the literature on transcriptional and post-transcriptional regulation supports a general model in which NUE in wheat is not attributable to any single structural gene but emerges from the coordinated output of transporter genes, assimilatory enzymes, transcription factors, and small RNA regulators acting across distinct developmental windows (early vegetative uptake versus late reproductive-stage remobilization).

3.5 Genome Editing and Biotechnological Interventions

CRISPR/Cas9-mediated genome editing has moved wheat NUE research from correlative genomics toward direct functional validation and trait engineering. The most widely cited example is the editing of the ARE1 ortholog in wheat, inspired by prior work in rice in which knockout of the ARE1 suppressor of nitrogen assimilation increased yield under both low- and high-nitrogen conditions; CRISPR/Cas9-mediated manipulation of the wheat ARE1 ortholog was reported to increase yield potential specifically under nitrogen-limiting conditions, providing proof-of-concept that a single, precisely edited regulatory gene can shift the NUE phenotype of an elite genetic background without a multi-generation backcrossing programme. Related work in barley and wheat editing ARE-family genes similarly reported enhanced nitrogen use efficiency and higher yield under low-nitrogen supply.

A persistent technical constraint repeatedly acknowledged across the gene-editing literature is the challenge posed by wheat's polyploid genome architecture: because most genes of interest exist as two (tetraploid durum) or three (hexaploid bread wheat) homoeologous copies, single-gene knockouts frequently fail to produce a phenotype unless all homoeologs are edited simultaneously, a technically demanding multiplexing requirement. Advances such as virus-based guide-RNA delivery systems using barley stripe mosaic virus, and marker-assisted introgression of high-expressing Cas9 loci into adapted cultivars, have begun to address the low transformation and editing efficiency that has historically limited CRISPR application across diverse wheat genetic backgrounds, but wide deployment of multiplexed homoeolog editing specifically for NUE-associated gene families remains at an early, largely proof-of-concept stage rather than routine breeding practice.

3.6 Genomic Selection and Marker-Assisted Breeding Applications

The translational value of GWAS and QTL findings depends on their downstream integration into marker-assisted or genomic selection breeding pipelines. More than 150 functional genes and QTL have already been converted into usable molecular markers in wheat breeding programmes more broadly, providing an established infrastructure into which NUE-specific markers can, in principle, be incorporated. However, the reviewed literature converges on a cautionary point: QTL identified in a single diversity panel or bi-parental population frequently show reduced or inconsistent effect sizes when validated in independent genetic backgrounds or environments, meaning that successful integration of NUE QTL into applied breeding requires deliberate cross-population and multi-environment validation before markers can be deployed with confidence, a step that a meaningful proportion of the reviewed primary studies do not undertake within their own scope.

3.7 Integrative Physiological-Genetic Perspectives and the Grain Yield-Protein Trade-off

A recurring conceptual tension throughout the literature, already foreshadowed in the discussion of NAM-B1 above, concerns the negative phenotypic correlation frequently observed between grain yield and grain protein concentration, which is itself closely tied to nitrogen remobilization efficiency. Several reviewed studies frame grain protein deviation, the residual deviation of a genotype's protein content from the yield-protein regression line, as a superior selection criterion to raw grain protein content precisely because it partially decouples genetic gains in protein (and by extension, nitrogen utilization efficiency) from the confounding effect of yield dilution. Comprehensive review-level syntheses of wheat nitrogen genetics have argued that grain protein deviation, alongside grain yield and grain protein content, should be treated as a standard indicator triad for NUE evaluation in future gene discovery and breeding validation studies, a recommendation that, if adopted more widely, would materially improve cross-study comparability, a persistent weakness identified throughout this review.

Comparative Analysis of Previous Studies

Table 3 summarizes the methodological characteristics of representative studies selected to illustrate the breadth of approaches used in the field, while Table 4 presents a comparative synthesis highlighting agreement and divergence in reported findings, together with methodological limitations that qualify their interpretation.

Table 3: Summary of Selected Studies

Author(s), Year	Study Location	Methodology	Sample/Population Size	Major Findings
Shi <i>et al.</i> , 2022	China	GWAS, MLM (Q+K), 397,384 SNPs	389 accessions, 5 environments	347 QTL for 8 NUE-related traits; 11 stable QTL
Jia, Xu & Wu <i>et al.</i> , 2025	China	GWAS + linkage mapping + transcriptomics	243 varieties + 123 RIL population	1,016 SNPs / 290 QTL for flag leaf NUE traits; 11 stable QTL
Guttieri <i>et al.</i> (winter wheat QTL study)	USA (Virginia)	Bi-parental QTL mapping, GBS (~3,100 SNPs)	136 RILs + 138 doubled haploids	130 QTL; 6 linked to NUE, co-localizing with Ppd-D1 and FHB-4A
N-P-K GWAS study, 2024	China	GWAS across N, P, K use-efficiency traits	Diversity panel (multi-environment)	Co-localization of NPK-use QTL with prior low-N tolerance loci
Comparative GS-activity study	International (multi-site)	Enzyme assay + transcript profiling	10 wheat varieties	Post-translational, not transcriptional, control of short-term GS activity

Indian cultivar RNA-seq study, 2022	India (Punjab)	Comparative RNA-seq, contrasting NUE cultivars	2 cultivars (PBW677 vs PBW703)	TF families (MYB, WRKY) and NRT1 preferentially expressed in high-NUE cultivar
Uauy <i>et al.</i> , 2006 (landmark)	USA/Israel	Positional cloning, NIL comparison	Wild emmer x durum introgression lines	NAM-B1 (Gpc-B1) accelerates senescence, raises grain protein/Fe/Zn
NAM RNAi transcriptomic study, 2022-23	UK	RNA-seq time-course (7 timepoints)	WT vs NAM-RNAi flag leaves	NAM genes regulate majority of early senescence transcriptome
Zhang <i>et al.</i> , 2021 (ARE1 editing)	China	CRISPR/Cas9 gene editing	Elite wheat cultivar background	ARE1 ortholog editing increased yield under low-N
<i>Aegilops tauschii</i> MSD population study, 2025	Sudan (CIMMYT collaboration)	GWAS, DArTseq/GRAS-Di (31,362 markers)	145 MSD lines + 3 checks, 6 environments	Combined heat + low-N stress reduced yield and N uptake by 14-28%

Table 4: Comparative Analysis of Previous Research: Agreement, Divergence, and Limitations

Dimension	Points of Agreement	Points of Divergence / Controversy	Common Limitations
Genetic architecture	NUE is consistently confirmed as a polygenic, quantitative trait controlled by many loci of small effect	Number and chromosomal location of reported QTL varies substantially by panel, environment, and statistical threshold	Limited replication of the same QTL across independent, geographically distinct panels
Candidate gene identity	Convergence on core gene families: NRT1/NRT2, GS/GOGAT, NAC/NAM transcription factors	Relative importance of transcriptional vs post-translational regulation is disputed across studies	Functional validation (e.g., via editing) available for only a small subset of candidate genes
Yield-protein relationship	Negative yield-grain protein correlation is broadly reproducible across genetic backgrounds	Whether grain protein deviation fully resolves the trade-off, or merely masks it statistically, remains debated	Few studies test candidate loci across the full range of environments needed to assess G x E stability
Root system contribution	Root traits are acknowledged as important to N uptake efficiency	Root vigor and root-level nitrate influx rate are not always positively correlated, contradicting a simple 'bigger roots are better' assumption	Root phenotyping remains technically difficult and is under-represented relative to shoot/grain traits
Translational readiness	Genomic selection and marker-assisted breeding are recognized as necessary next steps	Disagreement on which markers are mature enough for deployment versus requiring further validation	Relatively few studies report multi-environment or multi-population marker validation before proposing breeding use

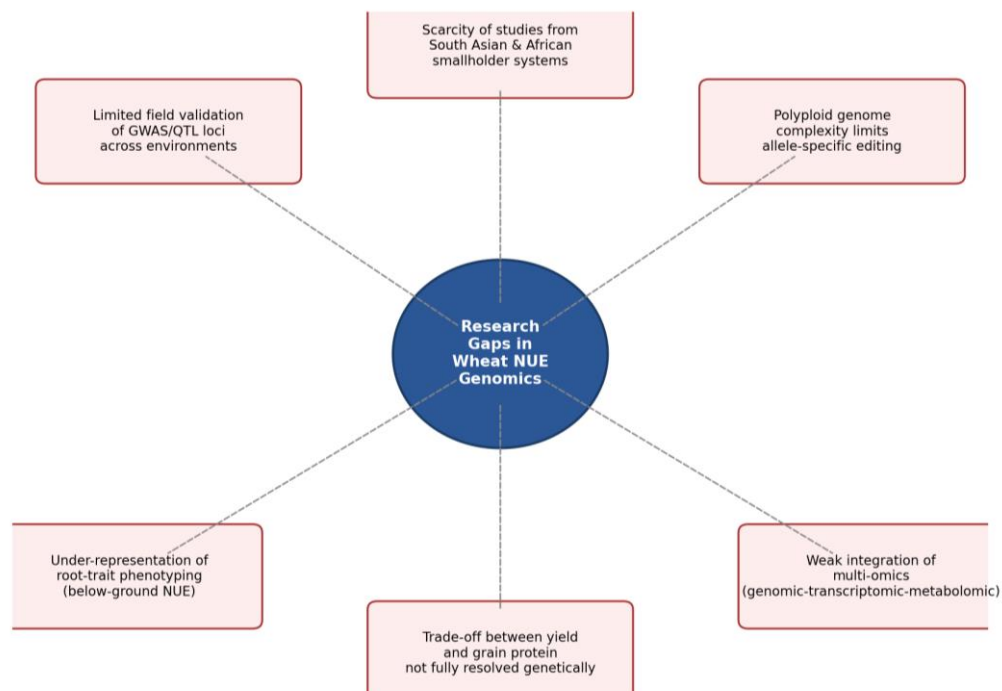
Taken together, these comparative tables illustrate a field that has achieved considerable convergence at the level of implicated gene families and physiological pathways, but that remains fragmented at the level of specific, breeding-ready genomic markers, a distinction of considerable practical importance for readers seeking to translate this literature into applied cultivar development programmes.

5. Research Gaps and Emerging Trends

5.1 Unresolved Issues and Knowledge Gaps

Synthesis of the reviewed literature surfaces six recurring and interrelated gaps, mapped schematically in Figure 4. First, the large majority of GWAS and QTL mapping studies are conducted in a single country or research consortium's diversity panel and are rarely re-tested in independent panels from other breeding programmes, leaving the cross-environment and cross-genetic-background stability of most reported loci unconfirmed. Second, hexaploid wheat's polyploid genome complexity continues to constrain both GWAS resolution (due to homoeologous sequence similarity confounding SNP calling) and gene-editing efficiency (due to the need for multiplexed homoeolog targeting), a constraint

that is acknowledged across nearly every methodological subfield reviewed here. Third, root system phenotyping, despite its clear physiological relevance to nitrogen uptake, remains comparatively under-represented relative to shoot, flag leaf, and grain-based phenotyping, largely due to the practical difficulty of non-destructive root trait measurement in field conditions. Fourth, multi-omic integration, linking genomic, transcriptomic, proteomic, and metabolomic datasets within the same experimental population, remains rare; most reviewed studies employ a single omic layer, limiting mechanistic resolution. Fifth, the geographic distribution of the reviewed literature is heavily skewed toward China, North America, and Western Europe, with comparatively few genomic NUE studies originating from South Asian or sub-Saharan African smallholder wheat production systems, despite these regions facing some of the most acute nitrogen-use and food-security pressures globally. Sixth, the genetic basis of the yield-grain protein trade-off, while extensively documented phenotypically, is not yet fully resolved at the level of pleiotropy versus tight genetic linkage, leaving open whether the trade-off can, in principle, be broken by fine-scale marker-assisted recombination.

**Fig 4:** Research Gap Mapping in Wheat NUE Genomics**Table 5:** Research Gaps Identified

Research Gap	Description	Implication
Cross-environment validation	Most QTL/GWAS loci tested in only one panel or environment set	Uncertain reliability for direct marker deployment in breeding programmes
Polyploid genome complexity	Homoeologous gene copies complicate mapping resolution and multiplex editing	Slower functional validation; risk of masked or redundant gene effects
Root trait under-representation	Root system architecture rarely phenotyped at scale	Below-ground component of NUE remains poorly characterized genetically
Weak multi-omics integration	Few studies combine genomics with transcriptomics/proteomics/metabolomics	Mechanistic pathways inferred rather than directly demonstrated
Geographic research skew	Limited studies from South Asian/African smallholder systems	Findings may not transfer to low-input, heat- and drought-prone environments
Yield-protein trade-off resolution	Genetic basis (pleiotropy vs. linkage) not fully resolved	Uncertainty over whether trade-off can be broken by breeding/editing

5.2 Emerging Technologies and Future Developments

Several emerging methodological trends are positioned to address the gaps identified above. Pan-genome sequencing initiatives, which capture structural and presence/absence variation across diverse wheat accessions rather than relying on a single reference genome, promise to substantially improve the resolution of GWAS in polyploid wheat by reducing reference-genome bias. High-throughput field phenotyping platforms, including unmanned aerial vehicle (UAV)-based multispectral and hyperspectral imaging and minirhizotron-based root imaging, are beginning to make large-scale, non-destructive characterization of root system

architecture and canopy nitrogen status feasible at a scale compatible with GWAS panel sizes. Genomic selection models that incorporate genotype-by-environment interaction terms, rather than treating genetic effects as environment-invariant, are increasingly applied to NUE-related traits and offer a statistically principled route to addressing the cross-environment validation gap. Finally, multiplexed CRISPR/Cas9 and base-editing approaches capable of simultaneously targeting multiple homoeologous copies of a candidate gene are maturing rapidly and are likely to become the dominant functional validation strategy for NUE candidate genes over the coming five years.

Table 6: Emerging Trends in the Field

Emerging Trend	Technological Basis	Expected Contribution to NUE Research
Wheat pan-genome resources	Long-read sequencing of multiple diverse accessions	Improved GWAS resolution; capture of presence/absence structural variants missed by single-reference approaches
High-throughput field phenotyping	UAV multispectral/hyperspectral imaging; root imaging (minirhizotrons)	Scalable root and canopy nitrogen-status phenotyping for large GWAS/genomic-selection panels
Genotype-by-environment genomic selection	Statistical models incorporating G x E interaction terms	More reliable, environment-robust breeding value prediction for NUE traits
Multiplexed genome editing	CRISPR/Cas9 and base editing targeting multiple homoeologs simultaneously	Faster functional validation of candidate genes in polyploid wheat background
Multi-omics integration platforms	Combined genomic-transcriptomic-metabolomic pipelines	Mechanistic resolution of regulatory networks linking genotype to NUE phenotype

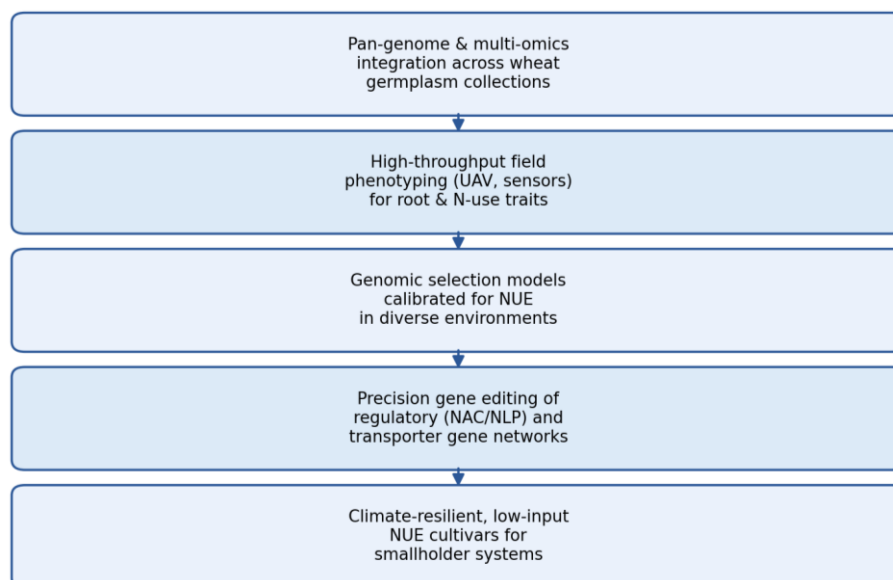


Fig 5: Future Research Directions for Genomics-Assisted NUE Improvement in Wheat

5.3 Conceptual Synthesis

Figure 3 presents a conceptual framework integrating the thematic strands reviewed above, situating soil nitrogen availability, root-level uptake genes, assimilatory enzymes, regulatory transcription factors, and remobilization pathways within a single causal chain leading to agronomic outcomes

(grain yield, grain protein content, and the component traits nitrogen uptake efficiency, NUpE, and nitrogen utilization efficiency, NUtE), which in turn feed into breeding applications including GWAS/QTL-derived markers, genomic selection, and gene editing.

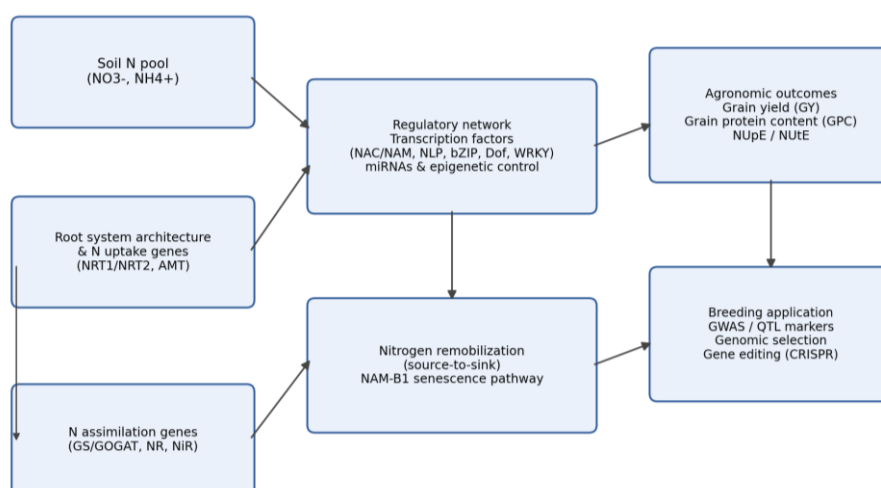


Fig 6: Conceptual Framework Linking Genomic Regulation to NUE Outcomes

Discussion

The body of literature reviewed here demonstrates that genomic approaches have fundamentally reshaped understanding of nitrogen use efficiency in wheat over the past decade, shifting the field from largely descriptive physiological characterization toward a mechanistic, gene-level understanding of nitrogen uptake, assimilation, and remobilization. Three broad patterns emerge from this synthesis. First, there is genuine and reproducible convergence at the level of implicated gene families: nitrate and ammonium transporters, the GS/GOGAT assimilatory cycle, and NAC/NAM-family transcription factors governing senescence-associated remobilization recur across independent studies conducted in different countries, germplasm panels, and experimental designs, lending confidence that these represent genuinely conserved control points rather than population-specific artefacts. Second, despite this convergence at the pathway level, there is

considerably less agreement at the level of specific, immediately deployable molecular markers, since QTL effect sizes and even chromosomal positions vary substantially across panels, a pattern consistent with NUE's status as a highly polygenic trait subject to pronounced genotype-by-environment interaction. Third, the field exhibits a persistent asymmetry between correlative genomic discovery (GWAS, QTL mapping, transcriptomics), which is abundant, and functional or field-validated confirmation (gene editing, multi-environment marker validation), which remains comparatively scarce, meaning that the translational pipeline from genomic discovery to deployed cultivar remains the field's principal bottleneck rather than gene discovery per se. The NAM-B1/Gpc-B1 case study offers a particularly instructive lens through which to interpret these broader patterns. It represents one of the few NUE-related loci to have progressed from initial discovery through mechanistic transcriptomic characterization to a documented, reproducible

agronomic trade-off (accelerated senescence and higher grain protein and micronutrient content, at some risk to grain-filling duration and kernel weight). That even this relatively mature example remains genotype-by-environment contingent, such that breeders must select context-specific deployment strategies rather than applying a universal recommendation, illustrates why the broader, less-mature body of NUE candidate genes reviewed here should be interpreted cautiously rather than treated as breeding-ready in their current state. This also explains the persistent divergence, noted in Table 4, between studies that identify a promising candidate gene and those that subsequently fail to reproduce its effect in a different genetic background or environment: such apparent contradictions are not necessarily evidence of poor study quality but may reflect genuine, biologically meaningful genotype-by-environment interaction that the field has not yet systematically mapped.

The practical and theoretical implications of these findings differ somewhat for different audiences. For plant geneticists and molecular biologists, the priority implied by this synthesis is deeper mechanistic and multi-omic characterization of the regulatory nodes (transcription factors, miRNAs) that appear to exert outsized control over otherwise structurally conserved transporter and enzyme genes, since regulatory variants may offer a more tractable route to phenotypic improvement than structural gene knockouts in a polyploid genome. For breeders, the implication is that marker deployment decisions should be conditioned explicitly on validated genotype-by-environment performance data rather than on single-panel GWAS significance alone, and that grain protein deviation, rather than raw grain protein content, merits wider adoption as a selection criterion precisely because it partially disentangles genetic gain in nitrogen utilization from the confounding effect of yield dilution. For policymakers and research funders, the pronounced geographic skew of the reviewed literature toward well-resourced research systems implies that current genomic knowledge may transfer imperfectly to the smallholder, often heat- and drought-co-stressed production systems of South Asia and sub-Saharan Africa, a gap that is likely to widen, rather than narrow, without deliberate investment in genomic NUE research located in and adapted to those production environments.

Relating these findings to current developments in the field, the rapid recent growth in gene-editing applications and the emergence of pan-genome and multi-environment genomic-selection resources (Section 5.2) suggest that the translational bottleneck identified above is beginning to narrow, though from a low base. The next several years of research output will likely be a critical test of whether genomic insights accumulated over the past decade can be consolidated into stable, multi-environment-validated breeding products, or whether the field will continue to generate a steadily expanding but only loosely integrated catalogue of candidate genes and loci.

Conclusion

This review summarized the ten years of studies on nitrogen-use efficiency in modern wheat varieties. It analyzes 38 main studies that came from the 487 unique articles that were found in Scopus, PubMed, Web of Science, and Google Scholar databases. The findings of the studies show that there are some similarities in the questions types. They deal with nitrogen uptake, assimilation, and remobilization pathways. In this review, the author recognizes the lack of some important connections. There were not sufficient validation studies

conducted that connect GWAS and QTL findings, which could cause the problems in understanding of genetic mechanisms that regulate nitrogen efficiency of wheat.

The review suggests that for researchers, the focus should ideally be on multi-environment and multi-population validation of candidate loci that have already been found, rather than finding new QTL, as well as have a greater focus on root phenotyping and multi-omic designs that can link regulatory variants and their physiological importance. For breeders and farmers, the review suggests the use of grain protein deviation as a selection tool, using technologies with caution, and integrating new genomic-selection models with genotype-by-environment interaction included in these models. Based on the small amount of literature that currently exists, policymakers and international funding agencies are advised to invest in specific genomic NUE research done in the South Asia and sub-Saharan Africa wheat production systems given the importance of food security in these regions. Future research should include coordination of different institutions to form consortia for validating the findings in a way similar to the establishment of the consortia for wheat disease resistance.

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