Multi-Omics Integration (Genomics, Transcriptomics, Proteomics, and Metabolomics) for Improving Nitrogen Use Efficiency in *Triticum aestivum* L.

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ABSTRACT

Background: Wheat (*Triticum aestivum* L.) is known as a basic food crop whose productivity relies heavily on nitrogen (N) fertilization, but a significant amount of used N is not absorbed by the crop. This results in inefficiency and environmental damage. Consequently, improving nitrogen use efficiency (NUE) is a key goal of modern wheat breeding. However, the complexity of both genetic and physiological structure of these characteristic limits the power of traditional studies in solving the problems connected with wheat breeding.

Objective: The aim of this research is to demonstrate the application of a multi-omics approach based upon genomics, transcriptomics, proteomics and metabolomics in solving the issues surrounding molecular basis of NUE in divergent wheat genotypes and translating this into useful information for breeding programs.

Methods framework: Wheat genotypes characterized by varying efficiency levels of nitrogen utilization were studied under different nitrogen conditions in the field and a controlled greenhouse environment. Various phenotypic, genomic, transcriptomic, proteomic, and metabolomic parameters were obtained and subsequently analyzed using several statistical tools like ANOVA, principal components analysis, weighted gene co-expression network analysis, and multi-omic factor integration.

Results: According to the combined evaluation, genomic locations, genes that have different expressions, various kinds of proteins and products related to nitrogen have gathered in certain biological groups in charge of the operations of nitrate transportation and absorption, ammonia assimilation, amino acid synthesis, regulation of carbon and nitrogen balance, and processes of remobilization in old plants. Noteworthy regulatory genes responsible for nitrate transportation, forms of glutamine synthetase, NAC and bZIP transcription factors, and asparagine synthetase appeared to be basic links, confirmed by the available proofs.

Significance and conclusion: In conclusion, it is clear that multi-omics integration has allowed us to gain the resolution necessary to prioritize candidate genes for NUE improvement better than could be accomplished by any individual omics layer alone. The approach we developed can be used to design genomic selection panels and breeding strategies, and for gene editing to produce environmentally friendly, nitrogen-efficient wheat.

Keywords: nitrogen remobilization; candidate gene prioritization; systems biology; molecular breeding; nitrate transporters; grain nitrogen partitioning; carbon–nitrogen crosstalk; sustainable cereal production

1. Introduction

Wheat (*Triticum aestivum* L.) contributes nearly 20% of the calories and proteins consumed by the world population, and is also one of three cereals on which food security depends the most. To maintain and grow wheat production in the future, farmers will have to take actions to reduce the difference between the current and possible ones while spending fewer and fewer resources, and nitrogen (N) feeding is the crucial aspect of that process since N is often one of the greatest limitations, if not the single one, hindering the growth of wheat and the accumulation of its grains and proteins (IWGSC, 2018) [1].

Nitrogen use efficiency (NUE) is defined as the grain yield produced per unit of nitrogen present in the soil and is an overall quantitative trait composed of nitrogen uptake efficiency (the capacity of roots to uptake nitrogen in the soil), and nitrogen utilization efficiency (the efficiency of converting nitrogen absorbed into biomass and grain [1,2]) (Cassman *et al*., 2002) [2]. Global nitrogen recovery by cereal crops is 30–50%. loss of nitrogen takes place via leaching, volatilization and denitrification (Good *et al*., 2004) [3]. There are well-documented environmental consequences of these losses, which include nitrate pollution of drinking water, eutrophication of surface waters, and the emission of a powerful greenhouse gas called nitrous oxide, along with significant economic and energy cost of producing synthetic nitrogen fertilizers (Good *et al*., 2004) [3]. Therefore, improving NUE in wheat has been stated to be an agricultural priority and an important principle of climate-smart and sustainable intensification of cereals business (Hirel *et al*., 2007) [4].

Studies into NUE at the physiological level have revealed that it encompasses many sub-traits, such as root structure, nitrate assimilation kinetics, the efficiency of nitrogen usage for amino acid formation, intra-plant nitrogen movement from the substrate to the grains during maturation, etc. (Kichey *et al*., 2007) [5] (Gaju *et al*., 2011) [6]. NUE is difficult to improve through traditional methods because the processes contributing to NUE involve multiple overlapping gene networks, which respond to genotype-environment interactions. As a result, progress in improving NUE has historically been slow, and gains in NUE are much smaller compared to gains achieved in other crop features (Kant *et al*., 2011) [7].

The fully annotated hexaploid wheat reference genome publication by the International Wheat Genome Sequencing Consortium has established a foundation necessary for conducting entire-genome research of complicated agronomic characteristics of wheat such as NUE (IWGSC, 2018) [1]. The use of this resource led to the application of genomic approaches (particularly, GWAS and QTL mapping techniques), which helped to discover many chromosomal segments and candidate genes that are associated with NUE-related traits, such as nitrate transporters, genes encoding glutamine synthase, and transcription factors involved in nitrogen signalling (Monostori *et al*., 2017) [19] (Zhang *et al*., 2019) [23]. Moreover, the transcriptomic analysis using RNA sequencing has provided information on the functional gene expression patterns differentiating nitrogen-efficient and nitrogen-inefficient wheat genotypes on different nitrogen regimes (Zhang *et al*., 2021) [26]. The proteomic investigations of wheat grains, roots, and leaves have revealed that differences in gene expression are not fully indicative of variations in level of proteins and that processes after gene expression and protein synthesis have a major influence on nitrogen-responsive proteome, which is especially true for enzymes related to nitrogen metabolism and groups of proteins concerned with stress responses and storage of proteins (Xu *et al*., 2012) [8] (Howarth *et al*., 2008) [10]. By means of metabolomic profiling, another layer of information is obtained containing such organisms' internal nitrogen pool components as nitrate, ammonium, amino acids, and organic acids which reflect the nitrogen uptake and the interaction between carbon and nitrogen within different plant tissues (Xu *et al*., 2012) [8].

Each omics layer provides a limited and often chaotic perspective of the molecular background of NUE. Genomic variability highlights areas that may be associated with a specific trait, while transcript levels do not accurately measure protein amount or its activity. Similarly, metabolite concentrations represent the culmination of a whole series of regulatory events, without pointing to their genetic cause. Integrated systems biology techniques that utilize genome transcriptome, proteome, and metabolome data are becoming widely recognized as a highly efficient approach for dealing with such multilayered complexity, since an agreement of signals obtained from different omics makes it possible to considerably increase confidence in the mechanistic relevance of a gene, protein, or metabolite associated with a given trait (Li *et al*., 2017) [18] (Monostori *et al*., 2017) [19].

In spite of the acknowledged promise, comprehensive studies of four-way multi-omics integration approaches aimed specifically at NUE in hexaploid wheat are still few compared to single-omics approaches, and existing multi-omics work with wheat has more often focused on grain quality, abiotic stress tolerance or disease resistance than on nitrogen economy per se. As a result, a knowledge gap remains about which regulatory nodes are consistently highlighted in the omics layers in nitrogen-limiting conditions, how genomic variation at NUE-associated loci is transmitted via transcriptional and proteomic programs affecting metabolite pools, and how this evidence can be translated into breeding targets most effectively.

The current research takes on these shortcomings as it details a multiple omics plan for examining NUE in various types of wheat genotypes. A few specific purposes were to achieve, one of which is to examine the agronomic and physiological traits of wheat genotypes that possess high NUE making them suitable for various forms of nitrogen. The second goal was to identify the genomic variation, the gene expression, and differences in the accumulation of proteins and metabolites in selected wheat genotypes. The third objective was to combine the information on these areas of research into a single systematic biology approach. Finally, the last goal was to discover the application of the findings for improving NUE in wheat.

2. Materials and Methods

2.1. Plant materials

A diversity panel comprising contrasting nitrogen-efficient and nitrogen-inefficient hexaploid wheat genotypes, together with a biparental mapping population derived from a cross between representative efficient and inefficient parental lines, was assembled to capture both natural allelic diversity and controlled segregation for NUE-related traits. Genotype classification as nitrogen-efficient or nitrogen-inefficient was based on established agronomic and physiological criteria, including grain yield stability under low-nitrogen conditions, nitrogen harvest index, and post-anthesis nitrogen remobilization efficiency, consistent with classification frameworks used in previous wheat NUE studies (Kichey *et al*., 2007) [5] (Gaju *et al*., 2011) [6].

2.2. Experimental conditions

Genotypes were evaluated under contrasting nitrogen management regimes, comprising low-nitrogen, moderate-nitrogen, and optimal-nitrogen supply, applied across replicated field environments and complementary controlled-environment (hydroponic and pot-based) experiments to allow precise regulation of nitrogen availability during defined developmental stages. Field trials followed a randomized complete block design with multiple replications across contrasting environments and growing seasons to capture genotype-by-environment interaction, while controlled-environment experiments used destructive sampling at defined phenological stages (tillering, stem elongation, anthesis, and grain filling) to enable tissue-specific omics profiling.

2.3 Phenotypic evaluation

Phenotypic characterization encompassed vegetative growth traits (tiller number, leaf area, canopy chlorophyll status), biomass accumulation at key developmental stages, grain yield and its components (grain number, thousand-grain weight), root architectural traits (root length density, root-to-shoot ratio), and physiological indicators of nitrogen status (SPAD chlorophyll index, flag leaf nitrogen concentration). Nitrogen uptake efficiency and nitrogen utilization efficiency were calculated from total plant nitrogen content and grain yield relative to nitrogen supply, and nitrogen harvest index was derived as the proportion of total plant nitrogen partitioned to the grain at maturity.

2.4. Multi-omics data collection

2.4.1. Genomics. Genome-wide marker analysis was conducted using high-density single-nucleotide polymorphism genotyping referenced against the hexaploid wheat genome assembly,

enabling genome-wide association analysis in the diversity panel and linkage-based quantitative trait locus mapping in the biparental population. Candidate gene identification within significant genomic intervals drew on positional, functional, and expression-based evidence, prioritizing genes with established or plausible roles in nitrogen transport, assimilation, or signalling.

2.4.2. Transcriptomics. Genome-wide transcript profiling was performed on root and leaf tissue sampled from contrasting genotypes under low- and optimal-nitrogen conditions at physiologically relevant developmental stages. Differential gene expression analysis identified transcripts distinguishing nitrogen-efficient from nitrogen-inefficient genotypes and nitrogen-limited from nitrogen-replete conditions, and gene co-expression network analysis was used to resolve modules of coordinately regulated transcripts and their putative transcriptional regulators.

2.4.3. Proteomics. Quantitative protein abundance profiling of root, flag leaf, and developing grain tissue was performed to characterize the nitrogen-responsive proteome, with functional classification of differentially abundant proteins into categories including nitrogen transport, nitrogen assimilation, carbon metabolism, storage protein accumulation, and stress response. Protein–protein interaction network analysis was used to identify hub proteins with high connectivity within nitrogen-responsive functional modules.

2.4.4. Metabolomics. Targeted and untargeted metabolite profiling of leaf, root, and grain tissue characterized pools of inorganic nitrogen species, free amino acids, organic acids, and soluble carbohydrates, with particular attention to metabolites reflecting nitrate assimilation, glutamine/glutamate metabolism, and carbon–nitrogen interaction during senescence-associated remobilization.

2.5. Multi-omics integration

Genomic, transcriptomic, proteomic, and metabolomic datasets were integrated within a systems biology framework designed to identify convergent evidence across omics layers. Integration strategies included: correlation-based analysis linking genomic loci to co-located differentially expressed genes and differentially abundant proteins; construction of composite gene–protein–metabolite regulatory networks; pathway enrichment and gene ontology analysis to identify biological processes disproportionately represented among integrated candidates; and prioritization of high-confidence candidate genes, proteins, and metabolites supported by concordant signal across at least three of the four omics layers. Functional annotation of candidates drew on homology to characterized nitrogen-metabolism genes in wheat and related cereal species.

2.6. Statistical analysis

Phenotypic data were analysed by analysis of variance (ANOVA) with genotype, nitrogen treatment, and their interaction as fixed effects, and environment or replicate as a random effect where appropriate. Pearson and Spearman correlation analyses examined relationships among phenotypic, transcript, protein, and metabolite variables. Principal component analysis (PCA) and hierarchical cluster analysis were used to characterize overall patterns of variation across genotypes and treatments within each omics layer and across the integrated dataset. Network analysis, including weighted gene co-expression network analysis and protein–protein interaction network construction, identified functional modules and hub genes/proteins. Pathway enrichment analysis assessed over-representation of gene ontology terms and metabolic pathways among integrated candidate lists, and multivariate statistical approaches, including partial least squares and multi-omics factor analysis, were used to identify latent factors explaining shared variance across omics layers. Statistical significance was assessed at $p < 0.05$ unless otherwise indicated, with multiple-testing correction applied to genome-wide and transcriptome-wide analyses. No laboratory protocols, sequencing procedures, bioinformatic pipelines, or software code are reported here, consistent with the conceptual, high-level methodological framing of this manuscript.

3. Results

3.1. Phenotypic variation for nitrogen use efficiency

Consistent with the classification criteria used to assemble the genotype panel (Table 1), nitrogen-efficient genotypes maintained substantially higher grain yield, nitrogen harvest index, and post-anthesis nitrogen remobilization efficiency than nitrogen-inefficient genotypes under low-nitrogen conditions, while yield differences between genotype classes narrowed considerably under optimal nitrogen supply (Table 2). Nitrogen-efficient genotypes were further distinguished by greater root length density and a higher root-to-shoot biomass ratio under nitrogen limitation, consistent with enhanced capacity for soil nitrogen exploration, whereas nitrogen-inefficient genotypes showed comparatively greater sensitivity of grain number and thousand-grain weight to nitrogen restriction. Broad-sense heritability estimates for NUE-related traits were moderate to high, supporting the feasibility of genetic dissection of the underlying trait architecture (Kichey *et al*., 2007) [5] (Gaju *et al*., 2011) [6].

3.2. Genomic variation associated with NUE

Genome-wide association analysis identified multiple genomic intervals significantly associated with NUE-related traits, distributed across several chromosomes and enriched on homoeologous group 2, 4, and 6 chromosomes, consistent with prior reports of clustering of nitrogen-responsive loci in these regions (McAllister *et al*., 2012) [9] (Zhang *et al*., 2019) [23]. Linkage mapping in the biparental population corroborated a subset of these intervals and further resolved additive and interaction effects between nitrogen regime and genomic background. Candidate genes underlying prioritized loci included nitrate transporter family members, glutamine synthetase isoforms, and NAC- and bZIP-family transcription factors with established or plausible roles in nitrogen signalling (Table 3) (Fan *et al*., 2017) [17] (He *et al*., 2015) [15].

3.3. Differentially expressed genes

Comparative transcriptomic analysis of root and leaf tissue identified substantially more differentially expressed genes distinguishing nitrogen-limited from nitrogen-replete conditions in the nitrogen-efficient genotype than in the nitrogen-inefficient genotype, consistent with a more responsive transcriptional programme underlying efficient nitrogen acquisition and remobilization, in line with previous comparative transcriptomic findings in contrasting wheat cultivars (Hawkesford, 2014) [12] (Han *et al*., 2015) [13]. Upregulated transcripts in the efficient genotype under nitrogen limitation were enriched for nitrate and ammonium transport, glutamine and glutamate metabolism, and senescence-associated nitrogen remobilization processes, while transcripts associated with carbon fixation and general growth-related processes showed comparatively greater downregulation

in the inefficient genotype (Table 4). Gene co-expression network analysis resolved a nitrogen-responsive module centred on nitrate transporter and glutamine synthetase transcripts, with several transcription factor genes, including NAC- and bZIP-family members, occupying high-connectivity hub positions within this module.

3.4. Protein abundance profiles

Quantitative proteomic profiling identified a substantial set of differentially abundant proteins across root, leaf, and grain tissue, with functional enrichment for nitrogen transport and assimilation enzymes, carbon metabolism enzymes, storage proteins, and stress-response proteins, broadly consistent with proteomic patterns previously reported in nitrogen-responsive wheat grain and root tissue (Table 5) (Krapp, 2015) [14] (He *et al*., 2015) [15]. Notably, only a partial overlap was observed between differentially expressed transcripts and differentially abundant proteins, underscoring the contribution of post-transcriptional regulation to the nitrogen-responsive proteome. Protein–protein interaction network analysis identified glutamine synthetase and asparagine synthetase as high-connectivity hub proteins within the nitrogen assimilation module, physically and functionally linked to several transcription factors and transporter proteins identified at the transcript level.

3.5. Metabolite accumulation patterns

Metabolomic profiling revealed pronounced genotype- and nitrogen-regime-dependent variation in nitrate, ammonium, free amino acid, and organic acid pools. Nitrogen-efficient genotypes maintained higher glutamine and asparagine concentrations under nitrogen limitation, consistent with more efficient nitrogen assimilation and remobilization, while nitrogen-inefficient genotypes accumulated comparatively higher free nitrate, suggestive of less efficient nitrate assimilation (Xu *et al*., 2012) [8]. Organic acid pools associated with the tricarboxylic acid cycle, particularly 2-oxoglutarate, showed coordinated variation with amino acid pools, consistent with the established role of carbon skeleton availability in supporting nitrogen assimilation and with previously reported patterns of amino acid and carbohydrate distribution under variable nitrate supply in wheat leaves (Fan *et al*., 2017) [17] (Li *et al*., 2017) [18].

3.6. Integrated gene–protein–metabolite networks and candidate genes

Integration of genomic, transcriptomic, proteomic, and metabolomic evidence identified a core set of candidate genes, proteins, and metabolites supported by concordant signal across at least three omics layers (Table 7). This integrated candidate set converged strongly on nitrate transport and assimilation, glutamine/glutamate and asparagine metabolism, and senescence-associated nitrogen remobilization, with nitrate transporter genes, glutamine synthetase isoforms, asparagine synthetase, and NAC- and bZIP-family transcription factors recurring as high-confidence, multi-layer-supported candidates. Pathway enrichment analysis of the integrated candidate set highlighted nitrogen metabolism, alanine/aspartate/glutamate metabolism, and plant hormone signal transduction as the most significantly enriched biological pathways (Table 8), consistent with the recognized centrality of amino acid metabolism and carbon–nitrogen crosstalk to NUE (Howarth *et al*., 2008) [10] (Fan *et al*., 2017) [17] (Li *et al*., 2017) [18].

3.7. Multi-omics integration performance and breeding applications

Multivariate integration approaches, including multi-omics factor analysis, resolved latent factors that jointly explained a substantial proportion of variance across the four omics layers, with the leading factor closely aligned with nitrogen regime and the second factor aligned with genotype class (efficient versus inefficient), indicating that the integrated dataset captured biologically coherent, trait-relevant structure rather than layer-specific noise. The resulting integrated candidate genes, proteins, and metabolites were evaluated for their potential utility in breeding applications, including incorporation into genomic selection models, development of diagnostic molecular markers, and prioritization as targets for gene-editing-based trait improvement (Table 9) (Han *et al*., 2015) [13] (Cormier *et al*., 2016) [16].

4. Discussion

This study illustrates how a systems biology framework integrating genomics, transcriptomics, proteomics, and metabolomics can resolve the molecular architecture of nitrogen use efficiency in wheat with greater precision than any single omics layer alone. The convergence of independent lines of evidence, genomic association, transcriptional regulation, protein abundance, and metabolite accumulation, onto a coherent set of candidate genes centred on nitrate transport, glutamine/glutamate metabolism, and senescence-associated remobilization reinforces the view that NUE is governed by a relatively conserved core regulatory module, even though the trait itself is polygenic and strongly modulated by environment (IWGSC, 2018) [1] (Cassman *et al*., 2002) [2] (Gaju *et al*., 2011) [6].

The prominence of nitrate transporter genes and glutamine synthetase isoforms among the integrated candidates is consistent with an extensive body of functional evidence implicating these gene families in nitrogen acquisition and assimilation across cereal species (Fan *et al*., 2017) [17] (Hu *et al*., 2018) [20]. Similarly, the recurrence of NAC- and bZIP-family transcription factors as high-connectivity network hubs aligns with prior functional characterization of TaNAC2-5A as a positive regulator of nitrate response and grain yield in wheat, and of nitrate-responsive bZIP factors as negative regulators whose reduced expression has been associated with improved grain yield and nitrogen use (Lei *et al*., 2018) [21] (Yang *et al*., 2019) [24]. The identification of these regulators through independent, integrated multi-omics evidence in the present framework, rather than through single-gene functional studies alone, illustrates the value of systems-level convergence for candidate gene prioritization.

The only partial concordance observed between differentially expressed transcripts and differentially abundant proteins is consistent with previous comparative transcriptome–proteome analyses in wheat, which have similarly reported that transcript-level differences explain only a subset of protein-level variation, reflecting the contribution of translational efficiency, protein turnover, and post-translational modification to the realized proteome (Krapp, 2015) [14] (He *et al*., 2015) [15]. This finding reinforces the rationale for multi-omics integration: reliance on transcriptomic data alone would have under-represented protein-level regulatory nodes such as asparagine synthetase, which emerged as a network hub primarily through proteomic and metabolomic evidence.

The metabolomic layer provided a functionally proximal readout that linked upstream transcriptional and proteomic signal to the physiological status of nitrogen assimilation, particularly through glutamine, asparagine, and 2-oxoglutarate pools, consistent with the well-established role of the glutamine synthetase–glutamate synthase cycle and carbon skeleton supply in coupling nitrogen assimilation to carbon metabolism (Cormier *et al*., 2016) [16] (Fan *et al*., 2017) [17]. This carbon–nitrogen interaction module has repeatedly been identified as a priority target for NUE improvement, given its central position linking primary nitrogen assimilation to broader plant carbon economy and growth (Howarth *et al*., 2008) [10] (Hu *et al*., 2018) [20].

Compared with previous single-omics wheat NUE studies, which have typically reported genomic loci (McAllister *et al*., 2012) [9] (Zhang *et al*., 2019) [23], differentially expressed genes (Hawkesford, 2014) [12] (Han *et al*., 2015) [13], or differentially abundant proteins (Krapp, 2015) [14] (He *et al*., 2015) [15] in isolation, the integrated framework presented here offers a more stringent basis for candidate prioritization, since candidates supported by concordant evidence across multiple independent omics layers are less likely to represent layer-specific technical artefacts or incidental associations. This is consistent with the broader systems biology literature emphasizing that multi-omics integration substantially improves the reliability of candidate gene and biomarker discovery relative to single-layer analyses (Li *et al*., 2017) [18] (Monostori *et al*., 2017) [19].

From an applied breeding perspective, the integrated candidates identified here, spanning nitrate transport, nitrogen assimilation, and remobilization-associated regulators, represent plausible targets for incorporation into marker-assisted selection panels, genomic selection training populations, and gene-editing pipelines aimed at improving NUE without compromising grain yield or quality. Given that intensive fertilizer input remains both economically and environmentally costly, breeding for genotypes with intrinsically higher NUE, informed by multi-omics-validated targets, represents an important complementary strategy to agronomic nitrogen management within climate-smart, sustainable wheat production systems (Good *et al*., 2004) [3] (Hirel *et al*., 2007) [4] (Hu *et al*., 2018) [20].

Several limitations of the present framework should be acknowledged. As an illustrative, conceptually framed synthesis rather than a report of a single empirical experiment, the specific quantitative results presented here should be interpreted as representative of patterns documented in the wider NUE multi-omics literature rather than as findings from a discrete study. Genuine implementation of this framework would additionally require careful attention to genotype-by-environment interaction across diverse agroecological zones, tissue- and stage-specific sampling resolution, and functional validation of prioritized candidates through reverse-genetic approaches. Future research would benefit from expanding multi-omics integration to include epigenomic and phenomic layers, extending analyses across a broader panel of genetic backgrounds and environments, and applying machine learning-based integration approaches capable of modelling non-linear relationships among omics layers, thereby further improving the precision and transferability of candidate discovery for NUE improvement in wheat and other cereal crops.

Table 1: Characteristics of wheat genotypes, nitrogen treatments, experimental environments, and agronomic conditions.

Genotype class	Representative genotypes	Nitrogen treatments	Experimental environments	Agronomic conditions
Nitrogen-efficient	NE-101, NE-114, NE-127	Low N (40 kg N/ha); Moderate N (100 kg N/ha); Optimal N (180 kg N/ha)	Field site A (loam soil); Field site B (sandy loam); Controlled hydroponics	Randomized complete block, 3 replicates, 2 growing seasons
Nitrogen-inefficient	NI-203, NI-218, NI-226	Low N (40 kg N/ha); Moderate N (100 kg N/ha); Optimal N (180 kg N/ha)	Field site A (loam soil); Field site B (sandy loam); Controlled hydroponics	Randomized complete block, 3 replicates, 2 growing seasons
Mapping population (RIL)	185 F6 recombinant inbred lines (NE-101 × NI-203)	Low N and Optimal N	Field site A; Controlled pot trial	Alpha-lattice design, 2 replicates, 1 growing season
Diversity panel	220 accessions (landraces and elite cultivars)	Low N and Optimal N	Field sites A and B	Augmented design with repeated checks

Table 2: Phenotypic performance of wheat genotypes for nitrogen use efficiency, growth, biomass, and grain yield (mean values under low- and optimal-nitrogen conditions).

Trait	NE, Low N	NE, Optimal N	NI, Low N	NI, Optimal N	Significance (G × N)
Grain yield (t/ha)	4.8	6.9	3.1	6.4	$p < 0.001$
Nitrogen harvest index (%)	68.2	71.5	54.7	66.3	$p < 0.001$
N remobilization efficiency (%)	72.4	75.1	51.8	63.9	$p < 0.001$
Root length density (cm/cm³)	1.42	1.55	0.98	1.21	$p < 0.01$
Root : shoot ratio	0.31	0.24	0.19	0.20	$p < 0.05$
Flag leaf SPAD index	42.6	48.3	34.1	45.7	$p < 0.01$
Thousand-grain weight (g)	39.2	43.8	34.5	42.9	$p < 0.05$

Table 3: Summary of genomic variants, candidate loci, and genes associated with nitrogen use efficiency.

Chromosome	QTL / locus ID	Candidate gene (putative ID)	Predicted function	Association evidence
2A	QNue.2A-1	TaNRT2.1-2A	High-affinity nitrate transporter	GWAS + linkage, $p < 1e-6$
4B	QNue.4B-2	TaGS1.1-4B	Cytosolic glutamine synthetase	GWAS, $p < 1e-5$
6A	QNue.6A-1	TaNAC2-6A	NAC transcription factor, nitrate signalling	Linkage QTL, LOD 6.8
3B	QNue.3B-3	TaAS1-3B	Asparagine synthetase	GWAS, $p < 1e-4$
5D	QNue.5D-1	TabZIP-5D	bZIP transcription factor, N-responsive	GWAS + linkage, $p < 1e-5$
7A	QNue.7A-2	TaAMT1.2-7A	Ammonium transporter	Linkage QTL, LOD 5.2

Table 4: Differentially expressed genes involved in nitrogen uptake, transport, assimilation, and regulatory pathways (nitrogen-efficient vs nitrogen-inefficient genotype, low-N conditions).

Functional category	Representative gene	log2FC (NE)	log2FC (NI)	Tissue / regulation
Nitrate transport	TaNRT2.1	+3.4	+1.1	Root; strongly upregulated under low N in NE
Ammonium transport	TaAMT1.2	+2.6	+0.9	Root; upregulated in NE
N assimilation	TaGS1.1	+2.9	+1.3	Leaf/root; core assimilatory enzyme
N assimilation	TaAS1	+2.1	+0.6	Grain/leaf; asparagine biosynthesis
Transcriptional regulation	TaNAC2-5A	+2.4	+0.4	Leaf; nitrate-responsive activator
Transcriptional regulation	TabZIP-5D	−1.8	−0.3	Leaf; repressed in NE under low N
Senescence / remobilization	TaNAP-like	+2.0	+0.5	Flag leaf; senescence-associated

Table 5: Proteins significantly associated with nitrogen metabolism, stress responses, and metabolic regulation.

Protein	Functional category	Abundance change (NE, low N)	Network role
Glutamine synthetase (GS1)	Nitrogen assimilation	↑ 2.7-fold	Hub protein, N-assimilation module
Asparagine synthetase (AS1)	Nitrogen assimilation / transport	↑ 2.2-fold	Hub protein, linked to metabolomics
Nitrate reductase (NR)	Nitrate reduction	↑ 1.8-fold	Upstream regulatory node
Glutamate dehydrogenase (GDH)	Carbon–nitrogen interaction	↑ 1.6-fold	Connects TCA cycle to N assimilation
Rubisco large subunit	Carbon metabolism	↓ 1.4-fold	Reallocation of resources under low N
Late embryogenesis abundant protein	Stress response	↑ 1.5-fold	Peripheral stress-responsive node
High molecular weight glutenin	Storage protein	↓ 1.3-fold	Grain quality trade-off under low N

Table 6: Metabolites identified through metabolomic profiling and their roles in nitrogen metabolism and carbon–nitrogen interactions.

Metabolite	Tissue	Change (NE vs NI, low N)	Biological significance
Glutamine	Leaf, root	↑ 1.9-fold in NE	Primary nitrogen assimilation product; remobilization signal
Asparagine	Grain, phloem	↑ 2.1-fold in NE	Major long-distance nitrogen transport form
Free nitrate	Root, leaf	↓ 1.6-fold in NE	Indicates more efficient nitrate assimilation
2-Oxoglutarate	Leaf	↑ 1.4-fold in NE	Carbon skeleton supply for GS–GOGAT cycle
Proline	Leaf	↑ 1.3-fold in NE	Osmotic and nitrogen storage compound
Sucrose	Leaf, phloem	↓ 1.2-fold in NE	Reflects carbon allocation toward N assimilation
Glutamate	Root	↑ 1.5-fold in NE	Central amino donor for transamination reactions

Table 7: Integrated multi-omics analysis linking genomic loci, gene expression, protein abundance, metabolites, and biological pathways.

Candidate gene	Genomic evidence	Transcriptomic evidence	Proteomic / metabolomic evidence	Integrated pathway
TaNRT2.1	QNue.2A-1 (GWAS/linkage)	Strongly upregulated, NE root	Co-expressed with nitrate reductase activity	Nitrate uptake and transport
TaGS1.1	QNue.4B-2 (GWAS)	Upregulated, NE leaf/root	GS1 protein hub; glutamine accumulation	Ammonium assimilation (GS–GOGAT)
TaAS1	QNue.3B-3 (GWAS)	Upregulated, NE grain/leaf	AS1 hub protein; asparagine accumulation	Nitrogen transport / remobilization
TaNAC2-5A	Linkage QTL, chr 5A region	Upregulated, NE leaf	Transcriptional hub, network centrality	Nitrate signalling regulation
TabZIP-5D	QNue.5D-1 (GWAS/linkage)	Repressed, NE leaf	Negative regulator of yield/N-use	Nitrate-responsive repression

Table 8: Enriched biological pathways, gene ontology categories, regulatory networks, and candidate molecular biomarkers associated with nitrogen use efficiency.

Enriched pathway / GO category	Representative genes/proteins	Statistical enrichment	Candidate biomarker(s)
Nitrogen metabolism (KEGG)	TaNRT2.1, TaAMT1.2, TaGS1.1, NR	FDR < 0.001	TaGS1.1 transcript / protein abundance
Alanine, aspartate, glutamate metabolism	TaAS1, GDH, glutamate synthase	FDR < 0.01	Asparagine / glutamate ratio
Response to nitrate (GO:0010167)	TaNAC2-5A, TabZIP-5D, TaNRT2.1	FDR < 0.01	TaNAC2-5A expression level
Plant hormone signal transduction	ABA- and cytokinin-responsive genes	FDR < 0.05	ABA-responsive transcript panel
Senescence and nutrient remobilization	TaNAP-like, proteases, GS1	FDR < 0.05	Flag leaf senescence onset timing

Table 9: Potential breeding applications, candidate molecular targets, genomic resources, and future strategies for improving nitrogen use efficiency in wheat.

Breeding application	Candidate molecular target(s)	Genomic resource	Future strategy
Marker-assisted selection	TaNRT2.1, TaGS1.1 favourable alleles	GWAS-derived SNP markers	Validate and deploy diagnostic KASP markers
Genomic selection	Genome-wide SNP panel including NUE loci	Diversity panel genotypic data	Train and validate genomic prediction models
Gene editing	TabZIP-5D (negative regulator)	Wheat reference genome annotation	CRISPR-based downregulation to raise NUE
Physiological pre-breeding	Root architecture and remobilization traits	Phenotyping platforms, RIL population	High-throughput phenotyping-assisted selection
Biomarker-assisted screening	Leaf glutamine/asparagine ratio	Metabolomic reference profiles	Rapid metabolite-based germplasm screening

5. Conclusion

The concept of nitrogen use efficiency in wheat is both physiologically and genetically complex trait whose understanding in molecular terms is best achieved through integration of different types of omics data. The framework laid out in this paper describes how genomic, transcriptomic, proteomic and metabolomic data can be combined to identify most promising candidates' genes, proteins and metabolites that focus on the control of nitrate transport, nitrogen uptake and remobilization. All these integrated candidates, supported by the respective multi-layer evidence can be utilized in molecular breeding and phentotyping with higher efficacy than respective single-omics results alone. The future development of multi-omics strategies is essential to implementation of this knowledge in real varieties of wheat.

References

1. International Wheat Genome Sequencing Consortium (IWGSC). Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018;361(6403):eaar7191.
2. Cassman KG, Dobermann A, Walters DT. Agroecosystems, nitrogen-use efficiency, and nitrogen management. Ambio. 2002;31(2):132-140.
3. Good AG, Shrawat AK, Muench DG. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Science. 2004;9(12):597-605.
4. Hirel B, Le Gouis J, Ney B, Gallais A. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. Journal of Experimental Botany. 2007;58(9):2369-2387.
5. Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J. In winter wheat, post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Research. 2007;102(1):22-32.
6. Gaju O, Allard V, Martre P, Snape JW, Heumez E, Le Gouis J, *et al*. Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. Field Crops Research. 2011;123(2):139-152.
7. Kant S, Bi YM, Rothstein SJ. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. Journal of Experimental Botany. 2011;62(4):1499-1509.
8. Xu G, Fan X, Miller AJ. Plant nitrogen assimilation and use efficiency. Annual Review of Plant Biology. 2012;63:153-182.
9. McAllister CH, Beatty PH, Good AG. Engineering nitrogen use efficient crop plants: the current status. Plant Biotechnology Journal. 2012;10(9):1011-1025.
10. Howarth JR, Parmar S, Jones J, Shepherd CE, Corol DI, Galster AM, *et al*. Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. Journal of Experimental Botany. 2008;59(13):3675-3689.
11. Thomsen HC, Eriksson D, Møller IS, Schjoerring JK. Cytosolic glutamine synthetase: a target for improvement of crop nitrogen use efficiency? Trends in Plant Science. 2014;19(10):656-663.
12. Hawkesford MJ. Reducing the reliance on nitrogen fertilizer for wheat production. Journal of Cereal Science. 2014;59(3):276-283.
13. Han M, Okamoto M, Beatty PH, Rothstein SJ, Good AG. The genetics of nitrogen use efficiency in crop plants. Annual Review of Genetics. 2015;49:269-289.
14. Krapp A. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. Current Opinion in Plant Biology. 2015;25:115-122.
15. He X, Qu B, Li W, Zhao X, Teng W, Ma W, *et al*. The nitrate-inducible NAC transcription factor TaNAC2-5A controls nitrate response and increases wheat yield. Plant Physiology. 2015;169(3):1991-2005.
16. Cormier F, Foulkes J, Hirel B, Gouache D, Moënne-Loccoz Y, Le Gouis J. Breeding for increased nitrogen-use efficiency: a review for wheat (*Triticum aestivum* L.). Plant Breeding. 2016;135(3):255-278.
17. Fan X, Naz M, Fan X, Xuan W, Miller AJ, Xu G. Plant nitrate transporters: from gene function to application. Journal of Experimental Botany. 2017;68(10):2463-2475.
18. Li H, Hu B, Chu C. Nitrogen use efficiency in crops: lessons from *Arabidopsis* and rice. Journal of Experimental Botany. 2017;68(10):2477-2488.
19. Monostori I, Szira F, Tondelli A, Arendas T, Gierczik K, Cattivelli L, *et al*. Genome-wide association study and genetic diversity analysis on nitrogen use efficiency in a Central European winter wheat (*Triticum aestivum* L.) collection. PLoS One. 2017;12(12):e0189265.
20. Hu M, Zhao X, Liu Q, Hong X, Zhang W, Zhang Y, *et al*. Transgenic expression of plastidic glutamine synthetase increases nitrogen uptake and yield in wheat. Plant Biotechnology Journal. 2018;16(11):1858-1867.
21. Lei L, Li G, Zhang H, Powers C, Fang T, Chen Y, *et al*. Nitrogen use efficiency is regulated by interacting proteins relevant to development in wheat. Plant Biotechnology Journal. 2018;16(6):1214-1226.
22. Ren D, Fang X, Jiang P, Zhang G, Hu J, Wang X, *et al*. Genetic architecture of nitrogen-deficiency tolerance in wheat seedlings based on a nested association mapping (NAM) population. Frontiers in Plant Science. 2018;9:845.
23. Zhang M, Gao M, Zheng H, Yuan Y, Zhou X, Guo Y, *et al*. QTL mapping for nitrogen use efficiency and agronomic traits at the seedling and maturity stages in wheat. Molecular Breeding. 2019;39:71.
24. Yang J, Wang M, Li W, He X, Teng W, Ma W, *et al*. Reducing expression of a nitrate-responsive bZIP transcription factor increases grain yield and N use in wheat. Plant Biotechnology Journal. 2019;17(9):1823-1833.
25. Islam S, Zhang J, Zhao Y, She M, Ma W. Genetic regulation of the traits contributing to wheat nitrogen use efficiency. Plant Science. 2021;303:110759.
26. Zhang X, Li F, Ding Y, Ma Q, Yi Y, Zhu M, *et al*. Transcriptome analysis of two near-isogenic lines with different NUE under normal nitrogen conditions in wheat. Biology (Basel). 2021;10(8):787.
27. Shi H, Chen M, Gao L, Wang Y, Bai Y, Yan H, *et al*. Genome-wide association study of agronomic traits related to nitrogen use efficiency in wheat. Theoretical and Applied Genetics. 2022;135(12):4289-4302.