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NITROGEN USE EFFICIENCY IN VEGETABLE CROPS: FROM NITROGEN ACQUISITION AND METABOLISM TO MOLECULAR REGULATION AND BREEDING

Vadada Vinay kumar[1] ,G. Madhuri[2] ,P. Syam Sundar Reddy[3]*, Kadiyala Prathima[4] ,Perumalla Srikanth[5] , Jaya Kumari[6] ,Vangapandu Thriveni[7]

[1]Assistant professor, Department of Horticulture, M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, 761211, Odisha, India. vadada.vinay@cutm.ac.in
[2]Assistant Professor, Department of Horticulture, School of Agriculture, Kaveri University, Gowraram Village, Wargal Mandal, Siddipet - 502279, Telangana, India. gmadhuri1810@gmail.com
[3]Principal Scientist, Dr. YSRHU, Citrus Research Station, Petluru, Tirupati, Andhra Pradesh. syamhort@yahoo.in,
[4]Ph.D. Research Scholar, Department of Vegetable Science, Dr. YSRHU-College of Horticulture, Anantharajupeta, Andhra Pradesh, India. Reddy, Prathimakadiyala07@gmail.com
[5]Assistant Professor, Agricultural Microbiology, School of Agriculture, Mohan Babu University, Tirupati-517102, India. Srikanth pcdgene@gmail.com,
[6]Assistant Professor cum Junior Scientist, Department of Horticulture (Floriculture and Landscaping), Nalanda College of Horticulture, Noorsarai, Nalanda Bihar Agricultural University, Sabour, Bhagalpur Bihar, 803113, kjaya010@gmail.com.
[7]Assistant professor, Department of Horticulture, M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, 761211, Odisha, India

ABSTRACT

Nitrogen (N) use efficiency (NUE) is a major determinant of sustainable vegetable production because high N demand, intensive fertilization and low recovery of applied N can increase production costs and environmental losses. In vegetable crops, NUE is a complex trait governed by coordinated processes operating from the soil–root interface to whole-plant N allocation and utilization. This review integrates current physiological, biochemical and molecular understanding of NUE in vegetable crops, with particular emphasis on the mechanisms that connect N acquisition with productive N utilization. The review first examines the conceptual components of NUE, including N uptake efficiency and N utilization efficiency and then synthesizes the roles of root architecture, nitrate and ammonium transporters, nitrate reduction, the GS/GOGAT cycle, amino-acid metabolism and carbon–nitrogen coordination. Particular attention is given to long-distance N transport, storage and remobilization, followed by emerging insights into nitrate sensing, NRT1.1-mediated signalling and NLP-dependent transcriptional regulation. Evidence from tomato, eggplant, cucumber, spinach, potato and other vegetable systems demonstrates that genotypic differences in NUE arise from coordinated variation in root traits, N transport, assimilation, photosynthetic capacity, metabolic plasticity and regulatory networks rather than from individual genes or enzymes. Transcriptomic, metabolomic and co-expression approaches are increasingly revealing candidate genes and regulatory modules associated with NUE, providing opportunities for molecular marker development, marker-assisted selection and genome editing. However, translation of molecular discoveries into field-level improvement remains constrained by the complexity of NUE, genotype × environment interactions and the need for robust phenotyping under contrasting N regimes. Future progress will depend on integrating physiological phenotyping, multi-omics, molecular breeding and precision N management to develop vegetable cultivars that maintain yield and quality with reduced N inputs.

KEYWORDS: Nitrogen use efficiency, Vegetable crops, Nitrogen metabolism, Nitrogen signalling and Molecular breeding

1. INTRODUCTION

Nitrogen (N) is an essential macronutrient governing plant growth and productivity through its roles in proteins, amino acids, nucleic acids, chlorophyll and enzymes, thereby linking N status with photosynthesis, carbon metabolism and biomass formation (Valenzuela, 2024; Hanif, 2023). Its importance is especially pronounced in intensively cultivated vegetables, where rapid growth and high marketable yield require substantial nutrient availability (Mauceri *et al.*, 2021; Aci *et al.*, 2021).

High N demand, however, can encourage excessive fertilizer use, increasing production costs and losses through leaching, runoff, volatilization, nitrification and denitrification (Valenzuela, 2024; Hanif, 2023). Potato provides a clear example of this challenge, with substantial applied N potentially remaining unavailable to the crop or being lost from the production system (Tiwari *et al.*, 2019). Improving N use efficiency (NUE) is therefore central to maintaining productivity while reducing unnecessary N inputs and environmental losses.

NUE represents an integrated outcome of N acquisition, assimilation, transport, utilization and remobilization. At the whole-plant level, it is commonly separated into N uptake efficiency (NUpE) and N utilization efficiency (NUtE), while N remobilization further influences the conversion of absorbed N into productive biomass and harvested yield (Lebedev *et al.*, 2021; Tiwari *et al.*, 2018). Its expression is also strongly affected by soil, climate, management and genotype × N interactions (Hanif, 2023; Ulas *et al.*, 2024).

Root architecture is a major determinant of N acquisition, as demonstrated by enhanced N uptake following grafting of watermelon onto vigorous pumpkin rootstock (Nawaz *et al.*, 2017). At the molecular level, contrasting tomato genotypes differ in nitrate storage, transport and remobilization, with SlNRT2.7 associated with superior NUE and NUtE under N limitation (Aci *et al.*, 2021). Transcriptomic and metabolomic studies further show that efficient N utilization involves coordinated changes in N metabolism, carbon metabolism and stress responses in spinach and potato (Joshi *et al.*, 2020; Xie *et al.*, 2024).

N-responsive regulatory networks provide another level of control. CsNLP1 links nitrate responses with N uptake and assimilation in cucumber (Li *et al.*, 2024), while WRKY33-associated networks contribute to high-NUE responses in eggplant (Mauceri *et al.*, 2021). Co-expression analysis has also identified NUE-associated candidate genes in cucumber, including NRT1.1 and other transport- and signalling-related genes (Ma *et al.*, 2024). These advances, together with molecular breeding and genome-editing resources available for vegetables, create opportunities to combine physiological selection with targeted genetic improvement (Lebedev *et al.*, 2021; Cardi *et al.*, 2017).

This review integrates the physiological, biochemical and molecular processes underlying NUE in vegetable crops, from N acquisition and assimilation to sensing, signalling, transport, remobilization and utilization, with emphasis on genotypic variation and opportunities for breeding and biotechnology.

2. Nitrogen Use Efficiency in Vegetable Crops: Concepts, Components and Determinants

NUE describes how effectively available or applied N is converted into plant biomass or economically useful yield. Because its definition depends on the input, output and biological scale considered, fertilizer recovery, NUpE, NUtE and N harvest index should be treated as complementary measures rather than interchangeable indices (Lebedev *et al.*, 2021; Valenzuela, 2024). The harvested organ is particularly important in vegetables: leaves are the principal sink in leafy crops, whereas fruits or tubers require efficient N transfer and remobilization (Tiwari *et al.*, 2018).

2.1 N acquisition and NUpE

NUpE depends on root exploration, root architecture and coordinated nitrate and ammonium transport. Root length, volume and diameter were associated with NUE differences among leafy vegetables, while vigorous pumpkin rootstock improved N acquisition in watermelon (Ulas *et al.*, 2024; Nawaz *et al.*, 2017). Genetic variation for NUE under contrasting nitrate supply has also been demonstrated in eggplant, providing a basis for physiological and molecular characterization of efficient genotypes (Mauceri *et al.*, 2020).

2.2 N utilization and internal N economy

NUtE reflects the efficiency with which absorbed N is assimilated, transported, remobilized and converted into productive biomass. In tomato, coordinated regulation of nitrate assimilation, transport and remobilization distinguished the efficient genotype Regina Ostuni from UC82 under limiting nitrate supply (Aci *et al.*, 2021). In potato, N remobilization and N harvest index are important components of N efficiency because absorbed N must ultimately support tuber development (Gastal & Lemaire, 2002; Fageria and Baligar, 2005; Han *et al.,* 2015; Tiwari *et al.*, 2018). The multidimensional nature of NUE requires its partitioning into complementary components that distinguish N acquisition from the subsequent utilization and redistribution of absorbed N. The principal indices used to characterize these processes are summarized in Table 1.

Table 1. Major components and indices used to characterize nitrogen use efficiency in vegetable crops

Component/index	General expression	What it represents	Biological interpretation
Nitrogen use efficiency (NUE)	Yield or biomass / N supply	Overall efficiency of converting available N into useful plant production	Integrates acquisition and utilization
N uptake efficiency (NUpE)	Plant N uptake / N supply	Capacity to acquire N from the available N pool	Primarily reflects root acquisition and transport
N utilization efficiency (NUtE)	Yield or biomass / plant N uptake	Efficiency with which absorbed N is converted into biomass or yield	Reflects assimilation, metabolism and productivity
N recovery efficiency	Increase in plant N attributable to N input / N applied	Fraction of applied N recovered by the crop	Particularly useful for fertilizer-use assessment
N remobilization efficiency (NRE)	Remobilized N / N available for remobilization	Capacity to redistribute internal N from older/source tissues to sinks	Important during reproductive development and sink filling
Nitrogen harvest index (NHI)	N in harvested organ / total plant N	Proportion of absorbed N allocated to the harvested product	Indicates N partitioning efficiency
Agronomic efficiency (AE)	Yield increase / N applied	Yield benefit obtained per unit of applied N	Fertilizer-response indicator
Physiological efficiency (PE)	Yield increase / increase in plant N uptake	Productivity generated from additional absorbed N	Separates utilization from acquisition
Agronomic N efficiency	Depends on experimental definition	Relationship between yield response and available/applied N	Should always be reported with the exact formula used

2.3 Physiological and environmental determinants

NUE is expressed through interacting root and shoot traits, including biomass, leaf area, chlorophyll, photosynthesis, tissue N and N partitioning. Integrated phenotyping therefore provides a stronger assessment than any single trait (Hirel *et al.*, 2007; Tiwari *et al.*, 2020a; Ulas *et al.*, 2024). N stress also alters photosynthesis, amino-acid metabolism and N partitioning, accompanied by extensive tissue-specific transcriptional responses (Joshi *et al.*, 2020).

Genotype × N interaction is a major consideration because the relative performance of genotypes can change with N supply. This interaction has been demonstrated for growth, root morphology and leaf traits in leafy vegetables and for nitrate metabolism in tomato and eggplant (Ulas *et al.*, 2024; Aci *et al.*, 2021; Mauceri *et al.*, 2020). Consequently, NUE screening should include contrasting N environments.

2.4 Management and integrated NUE

Soil pH and texture, salinity, rainfall, irrigation, temperature, tillage, pests, diseases, crop rotation and crop nutritional status all influence N recovery and utilization (Jalpa *et al.*, 2021; Tei *et al.*, 2020; Hanif, 2023). Appropriate N source, rate, timing and placement can improve synchronization between N availability and crop demand, while genotype selection determines the plant's capacity to capture and utilize that N (You *et al.*, 2023; Hanif, 2023; Valenzuela, 2024). NUE is therefore best viewed as an integrated soil–plant trait linking N availability, root acquisition, transport, assimilation, photosynthesis, sink development and environmental conditions.

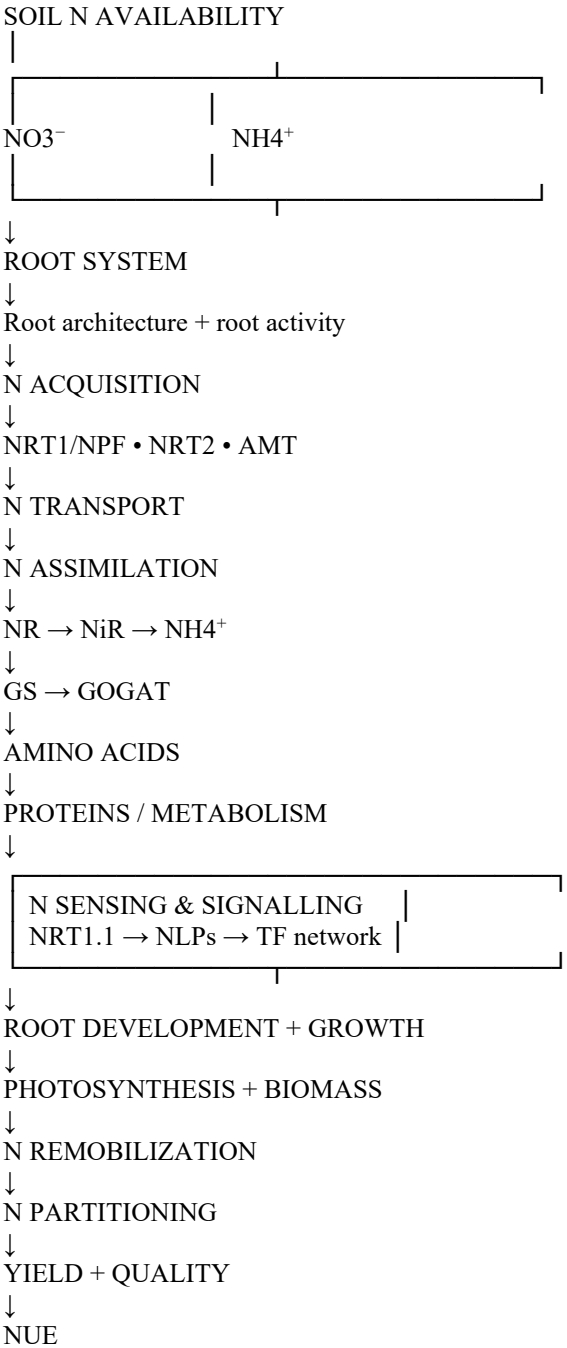


Figure 1. Integrated physiological and molecular framework governing nitrogen use efficiency in vegetable crops.

3. Molecular Architecture of Nitrogen Acquisition

Nitrogen acquisition begins with root–soil exploration and continues through membrane transport, intracellular handling and delivery to tissues where N is assimilated. Its contribution to NUE therefore depends on transporter activity, root architecture, N sensing and downstream metabolic capacity.

3.1 Nitrate and ammonium transport

Nitrate uptake is mediated mainly by NRT1/NPF and NRT2 systems Plett *et al.* (2010), whose relative contributions vary with nitrate availability and plant N status. In tomato, LeNRT2.3 contributes to nitrate acquisition and long-distance transport, demonstrating that high-affinity transport can influence both uptake and whole-plant N distribution (Fu *et al.*, 2015). In potato, higher NRT2.7 expression in a high-NUE genotype was associated with greater N concentration Wang *et al.* (2018), chlorophyll, yield and N-assimilation capacity under low N (Xie *et al.*, 2024). Cucumber transcriptomic analysis similarly identified NRT1.1 as a candidate NUE-associated gene, with a frameshift INDEL detected in a low-NUE line (Klaassen *et al.*, 2020; Ma *et al.*, 2024).

Ammonium acquisition is mediated largely by AMT proteins and must remain closely coupled with assimilation because excessive NH4+ accumulation can disturb metabolism. The AMT2 family in cassava displays tissue-specific expression and responsive expression under ammonium deficiency, illustrating the dynamic regulation of ammonium acquisition (Nacry *et al.*, 2013; Xia *et al.*, 2023). However, the available literature provides less vegetable-specific evidence for AMT-mediated NUE than for nitrate transport.

3.2 Root architecture–transporter coordination

Transporter capacity is constrained by the physical root system. Root length, branching and volume determine soil exploration, while transporter regulation controls the efficiency of N entry into root cells. N stress in spinach caused a strong root transcriptional response, including changes in NRT2.1 and NRT2.5, together with genes involved in amino-acid metabolism and regulatory pathways (Joshi *et al.*, 2020; Ruzicka et al, 2010). Physiological studies in leafy vegetables likewise associate NUE with root and leaf traits (Ulas *et al.*, 2024), while grafting studies demonstrate that improved root vigour can enhance both N acquisition and downstream assimilation (Nawaz *et al.*, 2017; Lynch, 2013). WRKY33-associated regulation in eggplant further links transcriptional control with root development and N uptake (Figure 4) (Nacry *et al.*, 2013; Mauceri *et al.*, 2021; Garnett *et al.*, 2009).

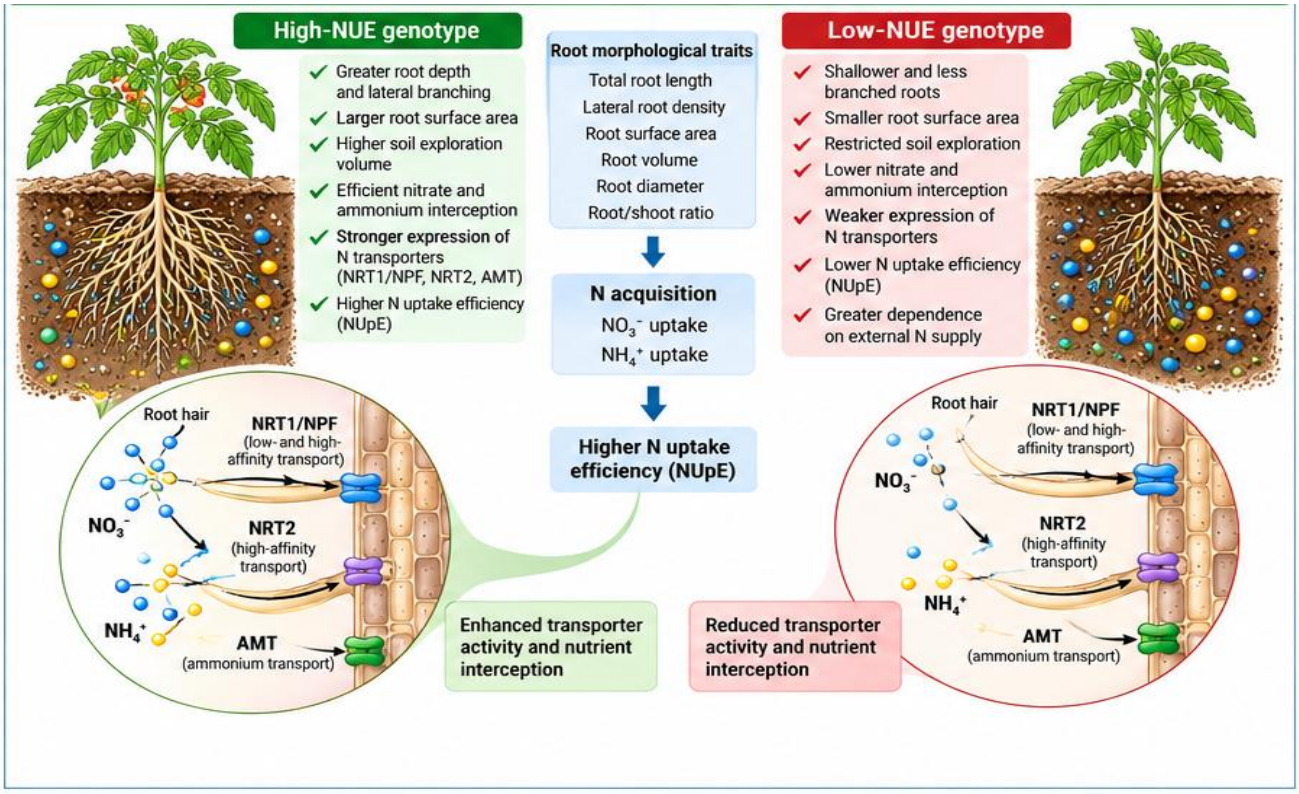


Figure 3. Root-system architecture and nitrogen acquisition as determinants of NUE in vegetable crops

3.3 Acquisition as a whole-plant process

Greater N uptake does not automatically increase NUE. Absorbed nitrate must be transported, assimilated and redistributed according to plant demand. Tomato genotypes differing in NUE show coordinated differences in nitrate storage, transport, remobilization and assimilation (Aci *et al.*, 2021). Similarly, molecular analyses indicate that NUE is governed by an interconnected network from uptake to assimilation and remobilization rather than by a single transporter

(Lebedev *et al.*, 2021). Thus, breeding for N acquisition should target allelic and regulatory variation that optimizes root exploration, transporter activity and coordination with downstream metabolism.
Molecular marker development also provides a route from mechanistic discovery to selection. A CAPS marker associated with the nitrate reductase gene in potato illustrates how variation in N-metabolism genes can be translated into marker-assisted selection (Tiwari *et al.*, 2019).

4. Intracellular Nitrogen Assimilation and Metabolic Integration
Nitrogen assimilation converts absorbed inorganic N into organic compounds required for protein synthesis, chlorophyll formation, nucleic-acid metabolism and growth. The pathway links nitrate and ammonium acquisition with carbon metabolism and depends on coordinated nitrate reduction, ammonium assimilation and organic N synthesis (Tiwari *et al.*, 2018; Lebedev *et al.*, 2021).
4.1 Nitrate reduction and GS/GOGAT assimilation
Nitrate reductase (NR) reduces nitrate to nitrite, nitrite reductase (NiR) converts nitrite to ammonium and the GS/GOGAT cycle incorporates ammonium into glutamine and glutamate. Their contribution to NUE depends on coordination across the pathway rather than on isolated enzyme activity (Tiwari *et al.*, 2018; Javed *et al.*, 2022). Under nitrate limitation, the efficient tomato genotype Regina Ostuni maintained nitrate-assimilation capacity more effectively than UC82, while high-NUE potato also showed stronger NR, GS and GOGAT activity under low N (Aci *et al.*, 2021; Xie *et al.*, 2024; Sun *et al.*, 2015).

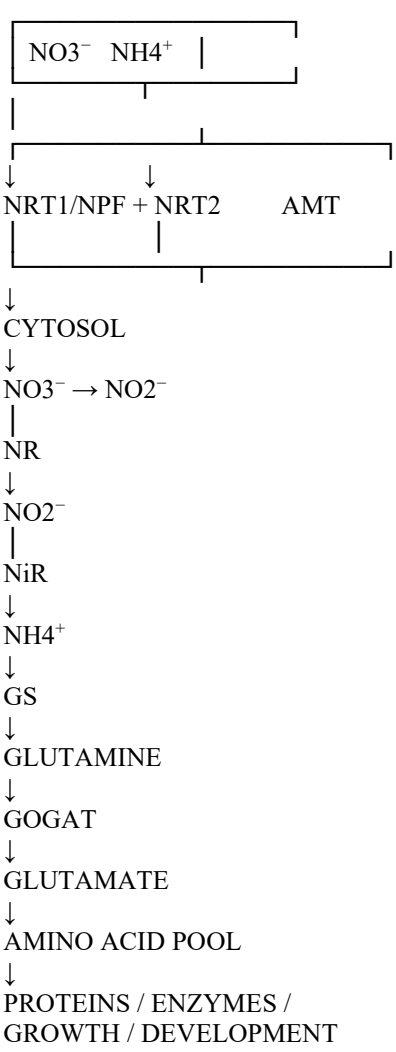


Figure 2. Molecular pathway linking nitrogen acquisition and intracellular nitrogen assimilation in vegetable crops.

Nitrate and ammonium are acquired through specialized transport systems and subsequently enter nitrate reduction and ammonium assimilation pathways. The GS/GOGAT cycle connects inorganic N assimilation with carbon skeleton availability and amino-acid metabolism.
4.2 Integration with carbon metabolism
GS/GOGAT activity requires carbon skeletons and reducing power, making C–N coordination fundamental to NUE Lea & Miflin (2003). In eggplant, N-efficient genotypes showed coordinated changes in amino-acid, soluble-sugar and

secondary-metabolite pathways under N limitation, while potato showed altered carbohydrate metabolism alongside stronger N transport and assimilation responses (Mauceri *et al.*, 2022; Xie *et al.*, 2024; Xu *et al.*, 2012). Efficient N assimilation therefore depends on maintaining an appropriate balance between N supply, carbon availability and growth demand (Masclaux-Daubresse *et al.*, 2010).

4.3 Amino-Acid Metabolism and Photosynthetic Integration

Glutamine and glutamate provide N for synthesis of other amino acids, including aspartate, asparagine and alanine, which participate in N transport, storage and remobilization. N-efficient eggplant genotypes displayed distinctive amino-acid profiles under N limitation, while tomato showed coordinated nitrate allocation and assimilation associated with improved N utilization (Mauceri *et al.*, 2022; Aci *et al.*, 2021; Xu *et al.*, 2012; Sun *et al.*, 2015; Giannino *et al.*, 2007).

N and photosynthesis are tightly interconnected because N is required for chlorophyll and photosynthetic proteins, whereas photosynthesis supplies carbon skeletons and reducing power for assimilation. High-NUE potato maintained higher chlorophyll, N accumulation and yield together with stronger NR, GS and GOGAT activity, while eggplant responses involved light-harvesting, ferredoxin–NADP reductase and antioxidant-related genes (Xie *et al.*, 2024; Mauceri *et al.*, 2021; Martínez-Andújar *et al.*, 2013).

4.4 Metabolic plasticity and whole-plant NUE

N-efficient plants adjust transporter expression, nitrate reduction, ammonium assimilation, amino-acid metabolism and carbon partitioning according to N availability. Tissue-specific responses in spinach demonstrate this plasticity, particularly in roots (Joshi *et al.*, 2020). Because high enzyme activity alone does not guarantee productive N utilization, assimilation must ultimately be linked with biomass formation, sink demand and remobilization (Lebedev *et al.*, 2021; Tiwari *et al.*, 2020b). NUE improvement therefore requires optimization of the complete assimilation network rather than manipulation of a single enzyme.

Table 2. Physiological, biochemical and molecular evidence for genotypic variation in nitrogen use efficiency across vegetable crops

Crop	Genotype/contrast	N condition	Major trait/evidence	NUE-associated response	Reference
Tomato	Regina Ostuni vs UC82	N limitation	Nitrate assimilation and N storage/remobilization	Efficient genotype-maintained N-assimilation capacity under restricted N	Aci *et al.* (2021); Ruzicka *et al.* (2010)
Eggplant	Contrasting genotypes	Contrasting/low N	Root and shoot N responses; transcriptomics	High-NUE genotypes showed coordinated regulatory and metabolic responses	Mauceri *et al.* (2021)
Eggplant	Contrasting accessions	N limitation	Metabolomics + transcriptomics	High-NUE genotypes displayed distinct metabolic profiles	Mauceri *et al.* (2022)
Cucumber	High- vs low-NUE lines	Low N	WGCNA/transcriptomics	NUE-associated modules and candidate genes including NRT1.1-related regulation	Ma *et al.* (2024)
Cucumber	CsNLP1 functional system	N response	N uptake and assimilation	CsNLP1 affected N uptake, assimilation	Li *et al.* (2024)

				enzymes and chlorophyll/biomass	
Spinach	Contrasting N responses	N stress	Root/leaf transcriptomics	Tissue-specific N-responsive genes and transcription-factor networks	Joshi *et al.* (2020); Xu *et al.* (2012)
Potato	Contrasting cultivars	Low N/contrasting N	Root traits, N uptake, assimilation, chlorophyll and productivity	Physiological and molecular traits differentiated N-efficient material	Tiwari *et al.* (2020a)
Potato	XS6 vs NS7	Low N	NR, GS, GOGAT, N transport and C metabolism	Higher coordination of N acquisition, assimilation and carbon metabolism in efficient cultivar	Xie *et al.* (2024)
Leafy vegetables	Multiple genotypes/species	Low N	Root length, root volume, root diameter and leaf traits	Root and shoot traits contributed to genotypic differences in NUE	Ulas *et al.* (2024); Li *et al.* (2015)

5. Long-Distance Nitrogen Transport and Remobilization

Once assimilated or transported as inorganic N, nitrogen must be distributed among roots, leaves and developing sinks. This is particularly important in vegetables where fruits, leaves or tubers may be spatially separated from the principal sites of N uptake and assimilation (Tiwari *et al.*, 2018; Lebedev *et al.*, 2021).

5.1 Xylem transport and nitrate storage

Root-absorbed nitrate may be assimilated locally, stored or loaded into the xylem for shoot transport. In tomato, the N-efficient genotype Regina Ostuni showed greater SlNRT1.5-associated shoot allocation, whereas UC82 showed stronger SlNRT1.8-associated root nitrate retention under prolonged limitation (Aci *et al.*, 2021). Short-term nitrate storage can buffer fluctuations in supply, but prolonged retention in roots may restrict delivery to expanding shoots and reproductive sinks. Thus, the timing of storage and mobilization is more informative than the expression of any single transporter (Masclaux-Daubresse *et al.*, 2010).

5.2 Organic N transport and phloem redistribution

After assimilation, glutamine, glutamate, asparagine and other amino acids become important forms for N transport and storage. Spinach transcriptomic responses show changes in glutamine/glutamate metabolism and asparagine-associated pathways in response to N availability (Joshi *et al.*, 2020). Phloem redistribution subsequently directs organic N from source tissues to developing sinks, with the balance depending on developmental stage and sink demand (Tiwari *et al.*, 2018; Sharifi *et al.*, 2007).

5.3 Remobilization and sink development

Remobilization recovers N from older tissues and redirects it to actively growing or reproductive organs, thereby contributing directly to NUtE. This process is particularly important when external N supply becomes insufficient during reproductive development (Tiwari *et al.*, 2018; Lebedev *et al.*, 2021). In tomato, efficient N utilization was associated with dynamic regulation of nitrate allocation rather than excessive root retention (Aci *et al.*, 2021). In potato, successful transfer of N into developing tubers is essential for converting absorbed N into harvestable yield (Xie *et al.*, 2024; Chen *et al.*, 2020; Ospina *et al.*, 2014; Tiemens-Hulscher *et al.*, 2014).

5.4 Transport as a determinant of NUtE

NUE therefore depends strongly on what happens after N enters the plant. Efficient nitrate loading, unloading, organic N transport and remobilization allow a given quantity of absorbed N to support more biomass or harvested product (Tiwari *et al.*, 2018; Javed *et al.*, 2022). Tomato and spinach evidence demonstrates that these processes operate as interconnected, tissue-specific networks (Aci *et al.*, 2021; Joshi *et al.*, 2020). Their coordination with N sensing and signalling provides the regulatory basis for dynamic N allocation (Masclaux-Daubresse *et al.*, 2010; Chen *et al.*, 2020).

6. Nitrogen Sensing and Signalling: The Regulatory Hub

Nitrogen sensing connects external N availability with transporter activity, root development, assimilation, carbon metabolism and growth. Rather than operating as a linear pathway, the N-signalling network integrates nutritional, developmental and environmental information to balance N acquisition with metabolic demand (Tiwari *et al.*, 2018; Javed *et al.*, 2022; Lebedev *et al.*, 2021).

6.1 NRT1.1 and nitrate–NLP signalling

NRT1.1/NPF6.3 combines nitrate transport with sensing and provides an early regulatory connection between external nitrate and downstream responses (Ma *et al.*, 2024). In cucumber, the NRT1.1 homologue was associated with contrasting NUE lines, including a frameshift INDEL in the low-NUE line, although functional validation remains necessary (Ma *et al.*, 2024; Marchive *et al.*, 2013; Wang *et al.*, 2018).

NLP transcription factors provide a major link between nitrate sensing and gene expression. Functional evidence from cucumber shows that CsNLP1 influences N uptake, total N, chlorophyll and the activities of NR, NiR, GDH, GOGAT and GS, together with nitrate-responsive genes (Li *et al.*, 2024; Ho & Tsay, 2010). This demonstrates how an upstream regulatory component can coordinate several downstream NUE processes.

6.2 Integration with carbon, calcium and growth signals

N sensing is integrated with cellular energy status, ion homeostasis and growth through signalling networks that include calcium-dependent responses and protein kinases (Javed *et al.*, 2022; Lebedev *et al.*, 2021). This coordination prevents N acquisition from exceeding the plant's metabolic capacity. Consequently, N responses are expressed not only through transporter activity but also through changes in root growth, carbon allocation and assimilation Marchive *et al.*, 2013 Gaudinier *et al.*, 2018.

6.3 Root development and feedback regulation

Root architecture is a major downstream output of N signalling because plants must adjust soil exploration to local N availability. Cucumber co-expression analysis identified NRT1.1 and other transport- and signalling-related genes within NUE-associated modules, illustrating the distributed nature of this regulation (Ma *et al.*, 2024). Feedback from internal N status also helps prevent excessive uptake or accumulation when assimilation and growth demand are limited (Javed *et al.*, 2022; Brooks *et al.*, 2019).

6.4 Signalling as a source of genotypic variation

Differences in signalling capacity can contribute to contrasting NUE phenotypes. CsNLP1 links nitrate perception with multiple N-assimilation processes in cucumber, while NRT1.1-associated variation has been identified among NUE-contrasting lines (Li *et al.*, 2024; Ma *et al.*, 2024). Thus, N sensing and signalling constitute a regulatory hub connecting N availability with acquisition, assimilation, transport, root development and growth and provide promising targets for genetic improvement when validated under relevant environments (Javed *et al.*, 2022; Lebedev *et al.*, 2021; Ma *et al.*, 2024).

7. Transcriptional Regulation and Regulatory Networks of Nitrogen Use Efficiency

NUE is expressed through coordinated gene activity controlling N acquisition, assimilation, transport, redistribution and growth. Transcriptomic evidence from eggplant, spinach, cucumber, tomato and potato indicates that genotypic differences are better understood as differences in regulatory coordination than as changes in isolated N-related genes (Mauceri *et al.*, 2021; Joshi *et al.*, 2020; Aci *et al.*, 2021; Tiwari *et al.*, 2020b).

7.1 Transcription factors as regulatory nodes

N limitation alters transcription-factor networks in addition to structural N-metabolism genes. In spinach, bHLH, MYB, WRKY and AP2/ERF families responded to N perturbation, indicating integration of N status with development and stress responses (Joshi *et al.*, 2020). In eggplant, WRKY33 was associated with high-NUE genotypes and genes involved in light harvesting, ferredoxin–NADP reductase and antioxidant activity, linking transcriptional regulation with N uptake and utilization (Mauceri *et al.*, 2021; Kiba *et al.*, 2018).

7.2 NLP-dependent regulation

NLP proteins provide an important nitrate-responsive regulatory layer. CsNLP1 suppression in cucumber reduced N uptake and total N and altered NR, NiR, GDH, GOGAT and GS activities, together with expression of nitrate-responsive genes (Li *et al.*, 2024). This positions NLP-dependent regulation between nitrate sensing and coordinated activation of N-acquisition and assimilation pathways Yanagisawa *et al.* (2004).

7.3 Co-expression and tissue-specific regulation

N responses are spatially and temporally regulated. Root and leaf transcriptomes in spinach showed distinct responses to N stress, while cucumber weighted gene co-expression analysis identified NUE-associated modules and candidate genes including NRT1.1, GLR22, GLR35 and UPS2 (Joshi *et al.*, 2020; Ma *et al.*, 2024). Such networks can reveal regulatory relationships that are not apparent from single-gene analysis.

7.4 From regulatory networks to breeding

The practical value of transcriptional studies lies in identifying stable regulatory nodes that can be linked with measurable NUE phenotypes. Molecular marker development for nitrate reductase in potato illustrates how N-related molecular variation can be translated into selection tools (Tiwari *et al.*, 2019). However, regulatory targets should be validated across N environments because altering an upstream regulator may affect growth, yield and quality as well as NUE (Figure 4) (Tiwari *et al.*, 2020b; Mauceri *et al.*, 2021; Ma *et al.*, 2024; Vidal *et al.*, 2020; Han *et al.*, 2015).

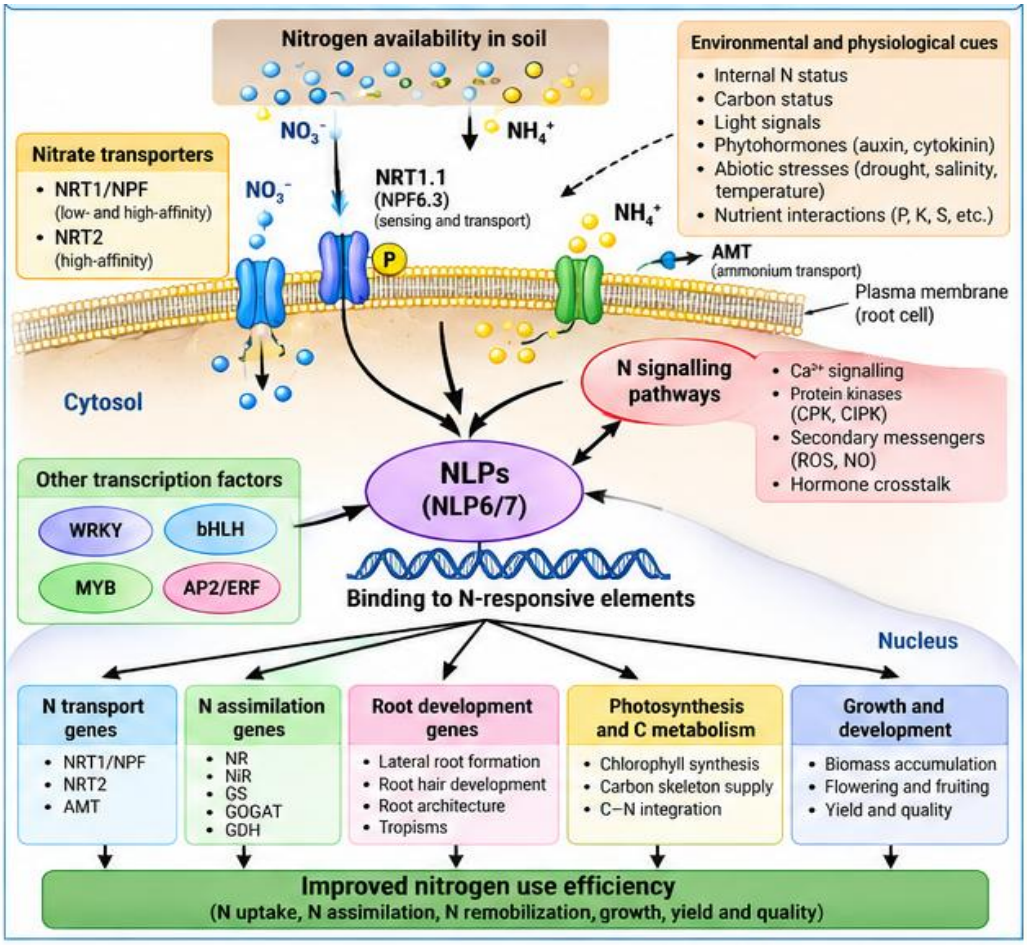


Figure 4. N sensing, signalling and transcriptional regulation of nitrogen use efficiency in vegetable crops

Table 3. Molecular components associated with nitrogen acquisition, assimilation, signalling and regulation of NUE in vegetable crops.

Crop	Gene/protein	Functional level	Reported relevance to NUE	Evidence type
Tomato	**LeNRT2.3** Klaassen *et al.* (2020)	Nitrate transport	Nitrate acquisition and long-distance transport	Functional molecular study
Tomato	**SlNR / SlNIR** Sun *et al.*, 2015	Nitrate assimilation	Maintenance of nitrate reduction under N limitation associated with efficient N response	Expression/physiological evidence
Eggplant	**WRKY33**	Transcriptional regulation	Associated with high-NUE phenotype, root development and N acquisition	Transcriptomic + functional evidence
Cucumber	**CsNLP1**	Transcriptional regulation	Influences N uptake, NR/NIR/GS/GOGAT/GDH-related responses	Functional study
Cucumber	**CsNRT1.1 homologue**	Nitrate sensing/transport	Candidate associated with high/low NUE variation	Transcriptomic + allele evidence
Cucumber	**GLR22 / GLR35**	Signalling	Candidate components of NUE-associated regulatory modules	WGCNA
Spinach	**NRT2.1 / NRT2.5**	High-affinity nitrate uptake	N-responsive root/leaf expression	Tissue-specific transcriptomics
Potato	**NR**	Nitrate assimilation	Candidate marker target for improving NUE	Molecular marker study
Potato	**GS/GOGAT-related system**	Ammonium assimilation	Associated with efficient N assimilation under low N	Physiological/molecular evidence

8. Integrated Physiological and Molecular Determinants of Genotypic Variation in NUE

Genotypic variation in NUE arises from coordinated differences in root architecture, N transport, assimilation, photosynthesis and regulatory responses rather than from a single trait (Tiwari *et al.*, 2018; Ulas *et al.*, 2024).

8.1 Root and shoot traits

Root length, volume and diameter contribute to soil exploration and N acquisition, while leaf area, chlorophyll and photosynthetic performance determine how effectively absorbed N supports growth (Ulas *et al.*, 2024). Enhanced root vigour can simultaneously increase N acquisition and downstream assimilation, as demonstrated in grafted watermelon (Nawaz *et al.*, 2017; Monostori *et al.*, 2017).

8.2 Metabolic and molecular determinants

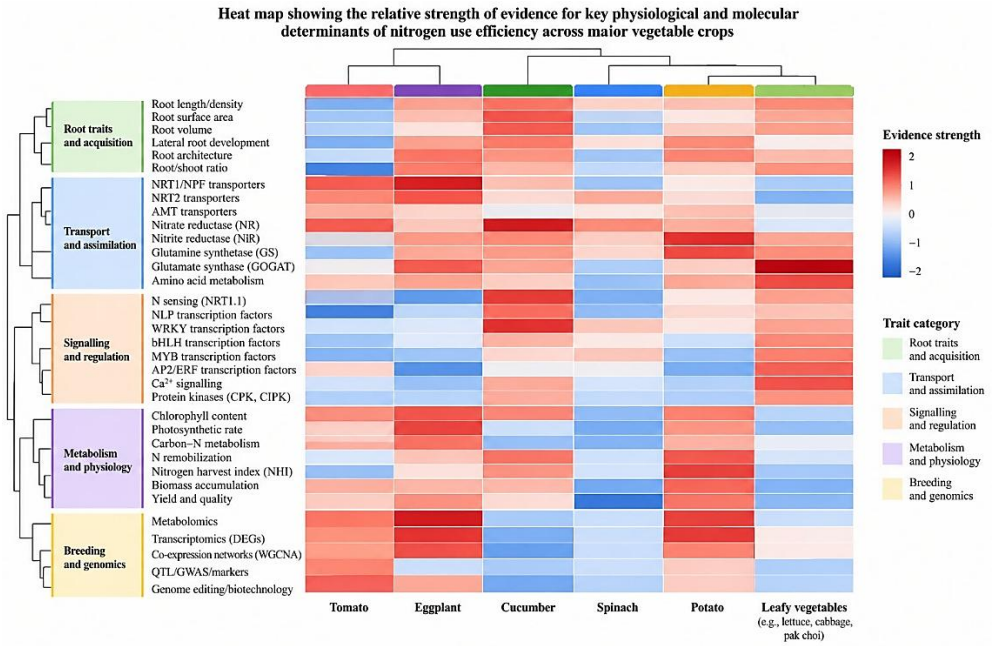
High-NUE potato maintained greater chlorophyll, N accumulation, yield and N-assimilation capacity under low N, accompanied by changes in N- and carbon-metabolism pathways (Xie *et al.*, 2024). Tomato genotypes likewise differed in nitrate transport and remobilization, particularly in the regulation of SlNRT1.5, SlNRT1.7, SlNRT1.8 and SlNRT2.7 (Aci *et al.*, 2021). Eggplant studies further associate NUE with coordinated transcriptional and metabolic responses, including WRKY33-related regulation (Mauceri *et al.*, 2021; Getahun *et al.*, 2020).

8.3 Integrated phenotyping for selection

Because NUE is multidimensional, selection should combine root traits, biomass, chlorophyll, N accumulation, NUpE, NUtE and molecular indicators. Integrating physiological phenotyping with transcriptomic and metabolic information can identify genotypes that maintain productivity under reduced N and provide more reliable targets for breeding (Tiwari *et al.*, 2020a; Ma *et al.*, 2024; Xie *et al.*, 2024).

Table 4. Physiological and metabolic traits associated with contrasting nitrogen-use-efficiency phenotypes in vegetable crops

Trait category	High-NUE phenotype	Low-NUE phenotype	NUE implication
Root architecture	Greater root exploration and/or favorable root traits	More restricted soil exploration	Improved N acquisition potential
Root length/volume	Higher or better-maintained under N limitation	Greater reduction under N stress	Supports NUpE
N transport	Strongly regulated transporter response	Less effective adjustment	Determines N acquisition
N assimilation	Maintained NR/NiR/GS/GOGAT activity under N limitation	Stronger decline in assimilation capacity	Supports NUtE
Chlorophyll	Better maintenance under low N	Greater decline	Supports photosynthetic C supply
Carbon metabolism	Better coordination with N assimilation	Greater metabolic imbalance	Provides carbon skeletons and energy
Amino-acid metabolism	Efficient redistribution/partitioning	Greater metabolic disturbance	Supports N utilization
N remobilization	Effective transfer from source to sink	Less efficient redistribution	Improves internal N economy
Growth	Maintained biomass under reduced N	Stronger biomass penalty	Overall NUE outcome
Yield	Maintained yield with lower N supply	Greater yield reduction	Agronomic relevance
Molecular response	Coordinated regulation of multiple N pathways	Less coordinated response	Network-level NUE



9. Breeding and Biotechnological Strategies for Improving NUE

NUE improvement requires integration of genetic variation, physiological screening and molecular approaches because the trait is controlled by multiple processes and is strongly influenced by genotype × environment interaction (Tiwari *et al.*, 2018; Tiwari *et al.*, 2020b; Thriveni *et al.*, 2024).

9.1 Conventional and marker-assisted breeding

Variation in root traits, N uptake, N utilization and productivity provides a foundation for selecting N-efficient germplasm. Molecular markers can increase selection efficiency; the CAPS marker associated with nitrate reductase in potato illustrates how N-metabolism variation can be translated into marker-assisted selection (Han *et al.*, 2015; Tiwari *et al.*, 2019). Transcriptomic and co-expression studies in eggplant, cucumber and potato can further provide candidate genes for integration with physiological selection (Hirel *et al.*, 2007; Mauceri *et al.*, 2021; Ma *et al.*, 2024; Xie *et al.*, 2024; Kumar *et al.*, 2025).

9.2 Transgenic and genome-editing approaches

Genetic engineering and genome editing offer opportunities to modify genes involved in N uptake, assimilation and remobilization. CRISPR/Cas technologies are particularly attractive because they permit targeted modification of candidate genes and regulatory regions, supported by expanding genomic resources in vegetable crops (Lebedev *et al.*, 2021; Cardi *et al.*, 2017; Tiwari *et al.*, 2020b; Nethra *et al.*, 2024). However, because NUE is highly interconnected, modification of a single gene may generate trade-offs in growth or productivity Hu *et al.* (2015), Tang *et al.* (2019), Zhao *et al.* (2018), .

9.3 Integrated breeding strategy

The most effective strategy is therefore to combine NUE phenotyping, molecular markers, genomic information and validation under contrasting N regimes. Biotechnology should complement rather than replace breeding, with the ultimate target being genotypes that maintain yield and quality through efficient N acquisition, assimilation, allocation and utilization under reduced N availability (Tiwari *et al.*, 2020b; Kumar et., 2024).

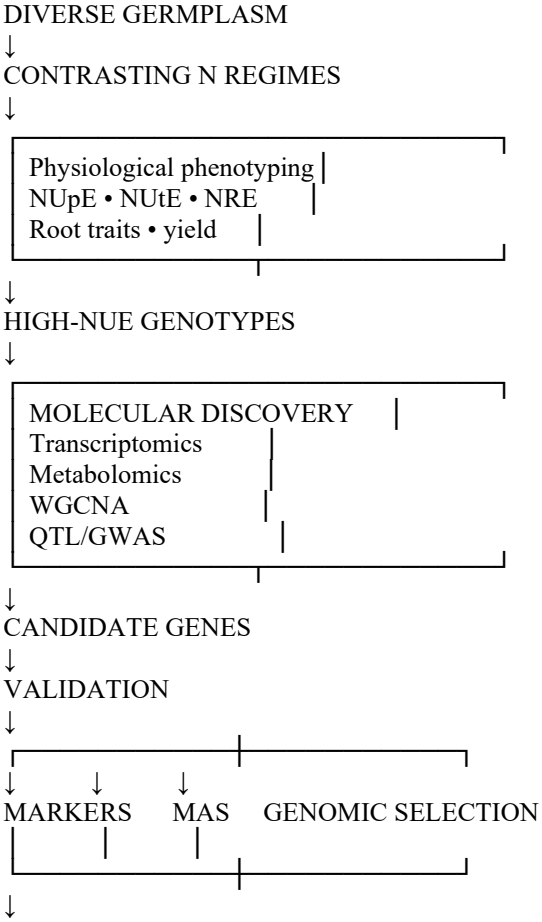


Figure 5. From physiological phenotyping to molecular breeding for improved nitrogen use efficiency in vegetable crops

Table 5. Breeding and biotechnological approaches for improving nitrogen use efficiency in vegetable crops

Approach	Primary objective	Examples/evidence	Major strength	Major limitation	Future NUE application
Germplasm screening	Identify natural NUE variation	Contrasting vegetable genotypes	Direct biological variation	Strong environmental influence	Foundation of breeding
Physiological phenotyping	Identify NUE-associated traits	Root traits, NUpE, NUtE, chlorophyll, yield	Links phenotype with function	Phenotyping can be laborious	Selection criteria
QTL mapping	Identify genomic regions	Root/NUE-associated QTL evidence	Locates genetic variation	Often polygenic	Marker development
GWAS	Identify marker–trait associations	Vegetable genomic resources	Exploits diversity	Population structure/environment	Candidate loci
Transcriptomics	Identify responsive genes	Eggplant, spinach, cucumber	Genome-wide response	Association requires validation	Candidate discovery
WGCNA	Identify co-expression modules	Cucumber	Network-level candidate discovery	Functional validation required	Regulatory-network breeding
Metabolomics	Identify metabolic signatures	Eggplant, potato	Captures functional phenotype	Expensive and complex	Biomarker discovery
Marker-assisted selection	Transfer favorable alleles	NR-related marker evidence in potato	Faster selection	Requires validated markers	NUE breeding
Genomic selection	Predict complex traits	Potential for polygenic NUE	Handles complex variation	Requires training populations	Accelerated breeding
Transgenic approaches	Modify N-related genes	Previous crop studies	Functional testing	Regulatory/acceptance issues	Gene validation
CRISPR/Cas genome editing	Modify candidate genes	Vegetable genome-editing studies	Targeted modification	Pleiotropy/off-target/validation concerns	Precision NUE breeding

10. CONCLUSION

Nitrogen use efficiency in vegetable crops is an integrated trait governed by coordinated N acquisition, transport, assimilation, sensing, allocation, remobilization and utilization. The available evidence shows that high NUE depends on the interaction of root architecture, transporter activity, metabolic capacity, photosynthesis and regulatory networks rather than on any single gene or process (You *et al.*, 2023; Lebedev *et al.*, 2021; Tiwari *et al.*, 2018). Integrating physiological phenotyping with transcriptomics, metabolomics, molecular breeding and genome editing can strengthen the identification and utilization of N-efficient germplasm. Ultimately, combining genetic improvement with precise N management provides the most practical pathway toward maintaining vegetable productivity while reducing fertilizer dependence and environmental N losses (Valenzuela, 2024; Hanif, 2023).

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