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REVIEW ARTICLE

Responses of cereals to nitrogen deficiency: Adaptations on morphological, physiological, biochemical, hormonal and genetic basis

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Abstract

Nitrogen (N) is a primary macronutrient essential for plant growth and development. Global nitrogen fertilizer consumption is approximately 120 million tons, with nitrogen use efficiency (NUE) ranging between 25 % and 50 %. Excessive use of nitrogen fertilizer poses significant risks to the environment and living organisms, highlighting the need to reduce fertilizer application, improve NUE, and sustain crop productivity. Sustainable agricultural practices emphasize minimizing fertilizer usage. Therefore, developing high-NUE crop varieties capable of maintaining yields under reduced nitrogen input is critical for ensuring food security and protecting ecosystem. A promising strategy involves investigating plant responses to varying nitrogen levels, particularly under low-nitrogen conditions. This review explores the morphological, physiological, biochemical, hormonal, and genetic changes in cereals subjected to low-nitrogen conditions. Morphological adaptations include alterations in root and shoot architecture, while physiological responses involve enhanced chlorophyll content, leaf nitrogen levels, and photosynthetic efficiency. Biochemical changes are characterized by increased activity of nitrogen uptake and assimilation enzymes, accompanied by hormonal shifts such as elevated auxin levels in roots. These traits provide a foundation for developing nitrogen-efficient crop varieties. Future research should prioritize breeding crops with enhanced tolerance to low-nitrogen conditions to improve NUE, grain quality, and yield potential.

Keywords

adaptations; food security; low nitrogen; nitrogen use efficiency; productivity; sustainable agriculture

Introduction

Cereal crops are vital for feeding the global population, providing more than half of the calories consumed by humans. Since the green revolution in the 1960s, the application of chemical nitrogen fertilizers to fertilizer-responsive and lodging-resistant "modern crop cultivars" of rice and wheat has significantly boosted food production. The use of nitrogenous fertilizers to enhance agricultural output has been a topic of discussion for over 50 years (1). Nitrogen fertilizer usage has increased from 10.8 million metric tons in 1960 to 109 million metric tons in 2019, with projections indicating a further rise to 249 million metric tons by 2050 (2). The higher utilization of N fertilizers compared to P and K fertilizers is attributed to their relatively low cost per nutrient unit, widespread availability, and the rapid yield response of modern crop cultivars to applied nitrogen. Approximately 60 % of global N fertilizer is used for the cultivation of the three major cereals: rice, wheat, and maize (3).

Cereal crops such as rice, wheat, and maize consume over 90 % of nitrogenous fertilizers globally. The total global rice production during 2022-2023 on a milled basis, was estimated at approximately 515 million . Wheat production exceeded that of rice, reaching approximately 793 million , while maize led as the top cereal, with an estimated production of 1159 million (4). In India, rice remained the predominant cereal crop, with an estimated production of 135.76 million cultivated over an extensive area of 47.83 million ha during 2022–2023 (5). Wheat ranked second, with a production of 112.74 million grown across 30.79 million ha (6). Maize, the third most significant cereal crop in India after rice and wheat, accounted for a production of 34.6 million over an area of 10.64 million ha (6).

Nitrogen, constituting approximately 1–5 % of a plant's dry mass, is the second most abundant nutrient in plant tissue. It is a fundamental component of amino acids, the building blocks of plant proteins, and plays a vital role in the growth and development of essential plant tissues. Additionally, nitrogen is an essential component of chlorophyll, the pigment responsible for enabling plants to use sunlight energy to convert water and carbon dioxide into sugars through photosynthesis. N also enhances root systems, significantly contributing to the absorption of water and nutrients (7). Its availability is the primary limiting factor for overall food production and protein content. The availability, uptake, and translocation of nitrogen influence critical physiological processes related to biomass production and grain yield.

In soil, nitrogen is predominantly present in a mobile form, with crops typically absorbing only 30–40% of the applied nitrogen (8). Consequently, more than 60 % of the soil nitrogen is lost through leaching, surface runoff, denitrification, volatilization, and microbial consumption. This inefficiency leads to low NUE without any significant yield improvements. Excess nitrogen escapes into the environment in reactive forms, such as nitrate (NO^-_3), ammonia (NH_3), and nitrogen oxides (NO_x and N_2O), which accumulate in natural ecosystems, causing nitrate pollution in surface and groundwater, altering biodiversity, and contributing to greenhouse gas emissions. In aquatic environments, excess nitrogen promotes algal blooms in coastal areas and freshwater lakes, depleting oxygen levels and harming aquatic organisms (9). Furthermore, nitrate accumulation in leafy and root vegetables poses health risks. High dietary nitrate intake can lead to endogenous nitrosation, which has been linked to thyroid disorders, certain cancers, neural tube defects in fetal development, and diabetes (10).

With the global population projected to exceed 9 billion by 2050, an estimated 60 % increase in food production will be required to meet demand (11). As the need for increased agricultural output grows alongside the urgency for environmental sustainability, transitioning to more judicious use of nitrogen fertilizers is imperative (12).

NUE refers to a plant's ability to produce grain yield per unit of nitrogen available in the soil. NUE is primarily governed by two processes: nitrogen uptake efficiency (NupE) and nitrogen utilization efficiency (NutE) (13). NupE represents the plant's ability to absorb nitrogen from the soil, while NutE reflects its ability to utilise absorbed nitrogen for grain yield production (14). NUE is influenced by nitrogen availability in the soil or through external inputs and depends on efficient nitrogen uptake, utilization, and remobilization to the grain by the end of the season. To mitigate nitrogen loss, minimize environmental pollution, and lower input costs, breeding crop varieties with higher NUE is critical. Research on genotypic variations under low and recommended N conditions, conducted during both seedling and maturity stage under controlled and field conditions, has identified high-NUE genotypes capable of maintaining high yield sustainability under low N conditions (15).

Enhancing crop plants for complex traits like NUE requires a thorough understanding of regulatory mechanisms, including nitrogen uptake, assimilation, and remobilization, as well as the performance of different genotypes, particularly under nitrogen-limited conditions.

This review article focuses comprehensively on cereal-specific traits and mechanisms, emphasizing their unique morphological, physiological, biochemical, hormonal, and genetic adaptations under low nitrogen conditions. By integrating these findings, it addresses knowledge gaps and provides targeted insights for breeding strategies that ensure high yield potential under reduced nitrogen input, a crucial component of sustainable agriculture.

Response of plants to low nitrogen stress

In natural agricultural fields, plants often encounter varying nitrogen levels, which can limit their growth due to factors such as surface runoff, soil erosion, rainwater leaching, gaseous losses, and microbial consumption. Under nitrogen deficiency, crops exhibit a reduced ability to capture and utilize resources, which is initially evident as a decline in the canopy's interception of photosynthetically active radiation (PAR). The reduction in PAR interception is primarily caused by decreased leaf expansion rates or tillering, leading to a reduction in the leaf area index (LAI).

The second major response to nitrogen deficiency is a reduction in radiation use efficiency (RUE), which occurs due to decreased leaf nitrogen content per unit leaf area. This decline in leaf nitrogen detrimentally impacts the rate of leaf photosynthesis. These responses represent a strategic trade-off for the plant, balancing between maintaining optimal resource capture and efficient resource use (16,17).

The significant effects of low nitrogen availability on plants, including changes in morphological, physiological, biochemical traits, hormonal regulation, and yield, are illustrated in Fig.1.The following sections outlines the morphological, physiological, biochemical, hormonal, and yield-related responses of cereal crops under low nitrogen conditions.

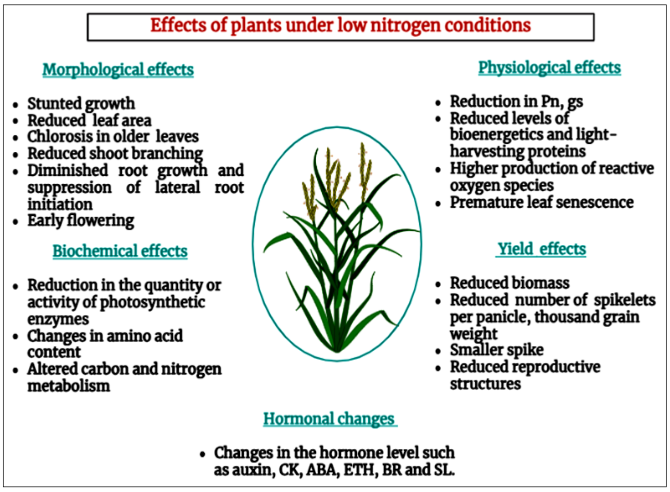

Fig. 1. Effects of plants under low nitrogen conditions. **Pn**- Photosynthetic rate; **gs**-Stomatal conductance; **CK**-Cytokinin; **ABA**- Abscisic acid; **ETH**- Ethylene; **BR**- Brassinosteroids; **SL**- Strigolactones.

Effect on morphological traits

Nitrogen plays a pivotal role in plant growth and development. Its availability is directly associated with vegetative development, and appropriate nitrogen application promotes growth and ultimately enhances yield (18). Conversely, insufficient nitrogen supply adversely impacts plant morphology, impairs growth, and results in reduced biomass (19). Nitrogen is vital for yield as it is integral to cell division, a process crucial for plant development. Disruption of cell division diminishes leaf area (20), which consequently leads to a decline in biomass accumulation (21). In cereals, typical morphological changes observed under low nitrogen conditions include decreased plant height, reduced tillering, a lower number of roots, and reductions in both shoot and root dry weight (22-26). Additionally, nitrogen deficiency leads to reduced leaf area, shorter leaf length, narrower leaf width (27), and yellowing of leaves (28).

Effect on physiological traits

Photosynthesis is a fundamental physiological process for plant growth and development, and its efficiency is significantly influenced by the availability of nitrogen. About 75 % of the nitrogen in plants is allocated to chloroplasts for the formation of the photosynthetic system, including thylakoid membranes and related enzymes. Insufficient nitrogen disrupts chloroplast structure (29), reduces chlorophyll levels, induces leaf yellowing (30), and ultimately impairs photosynthesis (31). Photosynthesis plays a crucial role in crop yield formation, supplying nearly 70% of the materials needed for yield production (26). In cereals, nitrogen deficiency reduces the photosynthetic rate due to significant reduction in chlorophyll content (chlorophyll *a* and *b*), stomatal conductance (31-34), Intercepted Photosynthetically Active Radiation (IPAR) (23), and leaf nitrogen content.

Nitrogen deficiency also leads to decreased chlorophyll concentration in leaves, which results in increased leaf reflectance in the green (approximately 550 nm) and red edge (700–720 nm) spectral ranges (35). Similarly, elevated leaf reflectance under nitrogen-deficient conditions was reported in sorghum (22). Chlorophyll fluorescence is a valuable tool for evaluating the photochemical efficiency of leaves, and nitrogen deficiency adversely affects the chlorophyll fluorescence efficiency of plants (36, 37). In cereals, nitrogen stress reduces the maximum efficiency of PSII photochemistry (Fv/Fm), inhibits electron transport, and decreases the quantum yield (38, 39, 33). Additionally, the tricarboxylic acid (TCA) cycle is suppressed under low nitrogen conditions (27).

Nitrogen deficiency also impairs photosynthesis-related enzymes and triggers biochemical stress responses, such as the overproduction of reactive oxygen species (ROS). These biochemical disruptions further exacerbate physiological impairments by limiting energy production and resource allocation for growth and yield formation. This dynamic interaction highlights how physiological impairments caused by nitrogen deficiency lead to biochemical imbalances, creating a feedback loop that poses challenges to plant development under low nitrogen conditions.

Effect on biochemical traits

Reactive oxygen species (ROS) are naturally produced during cellular processes such as photosynthesis and respiration. However, their concentrations can increase under stress conditions, including drought, salinity, extreme temperatures, and nutrient deficiencies. Under nitrogen-deficient conditions, the formation of ROS is observed (40). In cereals, low nitrogen availability leads to elevated levels of hydrogen peroxide (H_2O_2), which acts as both a marker of oxidative stress and a signal for physiological responses, along with an increase in malondialdehyde content (26). Nitrogen deficiency decreases the activity ratio of phosphoenolpyruvate carboxylase (PEPC) to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (41), adversely affecting photosynthesis in sorghum. It also impairs the enzymes of the ascorbate-glutathione cycle in both shoots and roots of barley (28).

The balance between carbon (C) and nitrogen (N) is essential for plant metabolic functions. Without sufficient carbon availability, the plant's ability to efficiently absorb and utilize nitrogen is compromised. Nitrogen significantly influences carbon fixation (42). A decline in nitrogen assimilation and storage reduces the plant's overall carbon fixation capacity (43). Under low nitrogen conditions, an increased leaf carbon-to-nitrogen ratio disrupts the glutamate cycle (44). Nitrogen plays a pivotal role in light-dependent processes that convert CO_2, water, and inorganic nitrogen into sugars, organic acids, and amino acids-key components for biomass accumulation. Low nitrogen availability also results in reduced levels of amino acids, such as leucine and phenylalanine, and a decline in protein content (28, 45, 46).

Effect on endogenous hormones

Under nitrogen-deficient conditions, hormonal responses in cereals are crucial for adapting to nutrient stress, as these hormones regulate key processes like growth, development, and NUE. Nitrogen signaling pathways are closely linked to several phytohormones, including auxin (AUX), abscisic acid (ABA), cytokinins (CKs), ethylene (ETH), brassinosteroids (BR), strigolactones (SLs), jasmonic acid (JA), and salicylic acid (SA) (47). These hormones interact to maintain a balance between the plant's nitrogen demand and acquisition. While CKs, ETH, and ABA typically play antagonistic roles, BR and AUX act synergistically to promote root elongation under nitrogen stress (48). During the grain-filling stage, the levels of hormones such as indole-3-acetic acid, CKs, ABA, and gibberellins decline under nitrogen-deficient conditions (49).

Effect on yield traits

Nitrogen drives critical physiological and metabolic processes, including nucleic acid metabolism, chlorophyll synthesis, and protein production, which enhance cell surface area, photosynthetic efficiency, and overall plant growth and yield (50). Despite its importance, nitrogen remains a major limiting factor for crop productivity, primarily due to the low efficiency of nitrogen fertilizer utilization, which constrains both the yield and quality of cereals. Yield reductions under low nitrogen conditions are attributed to a decrease in thousand-grain weight, the number of spikelets per spike (51), fewer grains per spike (27), and diminished spike and seed size (52). Limited nitrogen supply reduces the crop growth rate, leading to fewer reproductive structures and lower physiological attributes, ultimately resulting in reduced grain yield and its components (53).

Adaptations under low nitrogen stress in cereals

An essential adaptive strategy employed by cereals in response to nitrogen deficiency involves root elongation, which facilitates greater access to soil nutrients and nitrogen resources. Roots serve as the primary organ for nitrogen uptake in plants, playing critical roles in nutrient and water absorption, the synthesis of plant hormones, organic acids, and amino acids, as well as providing structural support to the plants. Adaptive changes in root morphology and physiological characteristics form the fundamental basis for efficient nitrogen utilization in plants. Root morphology and physiology are intricately linked to the acquisition of soil resources and the development of above-ground plant structures (54). The following sections discuss the morphological, physiological, and biochemical, hormonal adaptations, and genetic regulation observed in cereals under low nitrogen conditions, as illustrated in Fig.2.

Morphological adaptations

Morphological adaptations include changes in shoot and leaf development (52, 24) and alterations in root architecture (55, 56). Under conditions of low nitrogen availability, alterations in root architecture are observed, such as deeper root distribution (57), increased root length, root surface area, and root-to-shoot ratio (45, 25, 58), as well as an increase in lateral root count and root diameter (44). Lynch introduced the Steep, Cheap, and Deep (SCD) ideotype to enhance nitrogen and water acquisition in maize (59). This concept integrates root architectural, anatomical, and physiological traits to promote deeper rooting and improve nitrogen capture in leaching-prone environments. The term "steep" refers to the root growth angle and other architectural features that facilitate deeper rooting, while "cheap" pertains to traits that minimize the metabolic costs associated with soil exploration.

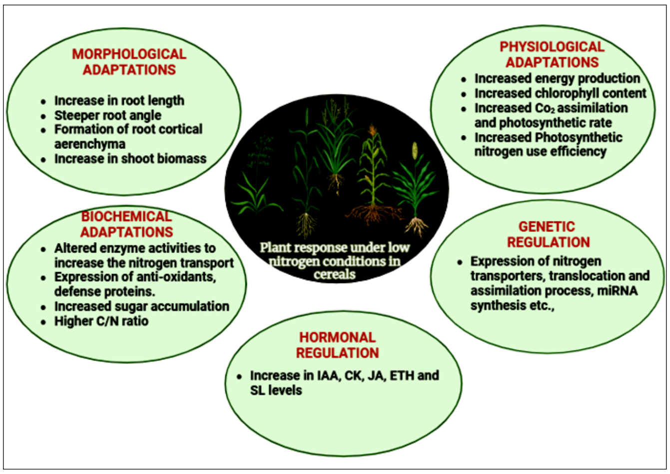

Fig. 2. Adaptations of cereals under low nitrogen conditions. **Co_2**- Carbondioxide; **C**- Carbon; **N**- nitrogen; **miRNA**- micoRNA; **IAA**- Indole acetic acid; **CK**- Cytokinin; **JA**- Jasmonic acid; **ETH**- Ethylene; **SL**- Strigolactones.

In cereal crops, the primary root is succeeded by seminal roots, followed by a series of nodal roots that develop near or above the soil surface. The primary root, which is essential for supplying water to the growing seedling, must grow vertically. It has been suggested that lateral roots branching from the primary root, along with seminal root axes and their lateral roots, may adapt plastically to local soil conditions. This plasticity could enhance the ability of plants to exploit variations in phosphorus and nitrogen availability, such as those arising from the mineralization of organic matter (60). Maize plants exhibit several adaptive changes by modifying root architecture, such as promoting the elongation of individual axial roots and enhancing lateral root growth, while simultaneously reducing the number of axial roots (61). Some maize plants also adjust by producing fewer crown roots, but with greater rooting depth (62). Furthermore, under nitrogen-deficient conditions, the angles of brace and crown roots become up to 18 degrees steeper (63). The formation of root cortical aerenchyma under low nitrogen conditions increases rooting depth and facilitates oxygen transport (64).

The key factors driving changes in root morphology are the differential alterations in the abundance of proteins involved in cell differentiation, cell wall modification, phenylpropanoid biosynthesis, and protein synthesis (65). Developing a robust root system architecture that integrates diverse root traits, such as nodal root growth, root hair length and density, root length density, root dry weight, lateral root percentage, root branching, root thickness, and root volume, offers a potential solution for enhancing nutrient uptake efficiency, particularly nitrogen uptake (66). Root nitrogen uptake can also be enhanced by nitrogen fixation, which in turn increases crop yield. The artificial introduction of nitrogen-fixing microorganisms has shown potential in boosting nitrogen uptake. The diverse microbial species, natural *nif* gene clusters, and engineering approaches to develop bacteria capable of delivering fixed nitrogen to cereal crops (67). Additionally, several rhizospheric microbial mechanisms were identified that influence root architecture by promoting the production of growth hormones such as auxins, cytokinins, and gibberellins (66).Morphological changes in the shoot include increased shoot biomass (57, 68). The enhanced root and shoot growth, reflected in larger biomass and a higher crop growth rate (CGR) under reduced nitrogen levels, contributes to greater grain yield, ultimately enhancing nitrogen use efficiency (NUE) in nitrogen-efficient varieties (68). In C4 plants, the thinner cell walls of mesophyll cells improve mesophyll conductance, while the thicker cell walls of bundle sheath cells minimize CO_2 leakage, thereby enhancing its concentration. Sorghum leaves, when faced with nitrogen limitation, maintain the essential anatomical structure necessary for C4 photosynthesis through intracellular adjustments. Under decreasing leaf nitrogen content, the activity of phosphoenolpyruvate carboxylase (PEPC) declines more significantly than that of Rubisco, suggesting that sorghum plants adapt to nitrogen limitation by reducing nitrogen allocation to PEPC more than to Rubisco (41).

Physiological adaptations

Under low nitrogen conditions, plants enhance nutrient uptake and utilization by increasing nitrogen transport, assimilation, and the remobilization of compounds such as glycine, allantoin, and proteins, which are crucial for maintaining optimal growth and development (65). Additionally, plants inhibit transcription factors that negatively regulate nitrogen transport (44). The nitrogen transport and assimilation process are enhanced by increased production of energy and α-ketoglutarate through the promotion of the tricarboxylic acid cycle (32). Root morphological changes, such as the formation of root cortical aerenchyma, lead to increased leaf nitrogen content, leaf chlorophyll content, CO_2 assimilation, vegetative biomass, and photosynthetic rate (64). Traits such as photosynthetic nitrogen use efficiency, root oxidation activity, and the accumulation of non-structural carbohydrate (NSC) in the stem at heading, along with their remobilization during grain filling, were found to increase under low nitrogen conditions (57,68). Furthermore, specific rearrangements in nutrient element levels within tissues were observed, including more stable manganese (Mn) and copper (Cu) contents in the roots, and less reduction in root phosphorus (P), potassium (K), and calcium (Ca) contents (69). Delaying the onset of post-anthesis senescence may be a crucial trait for enhancing grain yield under low N conditions (70).

Biochemical adaptations

Nitrate uptake in plants is facilitated by high-affinity nitrate transporters (NRT2) and low-affinity nitrate transporter 1/peptide transporter family (NRT1/NPF), while ammonium absorption is regulated by ammonium/methyl ammonium transporters (AMTs). In rice, nitrogen uptake is enhanced under low nitrogen conditions through the expression of nitrate and ammonium transporters (32). Nitrogen transport can be further enhanced by altering the activities of enzyme such as nitrate reductase, glutamine synthetase, and glutamate synthase (56,71,69). To resist oxidative stress, crops like rice (72) and wheat (24) have developed antioxidant defense systems to detoxify ROS. Several molecules, including ROS and nitric oxide (NO), have been proposed to play a role in the signaling pathways that link stress perception to gene expression. A short-term rise in H_2O_2 levels in the roots could be involved in signaling mechanisms related to nitrogen deficiency (73). In response to low nitrogen stress, plants enhance the expression of antioxidant-related proteins, including glutathione and ascorbic acid (27, 45, 65, 74), defense proteins (75), and phenolic metabolites (45), while also reducing malondialdehyde content (76). As fiber and starch are primarily composed of polysaccharides, an increase in thousand-grain weight (TGW) is linked with enhanced sugar accumulation. Under field conditions, nitrogen deprivation reduced the number of grains per spike and resulted in fewer grains per unit area, allowing more nitrogen to be available for each grain. These factors contributed to the higher carbon-to-nitrogen ratio in low nitrogen plants, promoting sugar synthesis and transport to the grain (27).

Hormonal adaptations

Plants activate complex hormone signaling networks to adapt and optimize resource allocation under low nitrogen conditions. The Ethylene Response Factor (ERF) transcription factor gene family and genes related to indole-3-acetic acid were specifically expressed, where they regulate root growth and morphology to help plants tolerate low-N stress (58). In response to signals indicating low nitrogen levels in the shoot, there is an increase in auxin transport from the shoot to the root. Low nitrogen conditions regulate genes related to plant hormones, promoting the polar transport of auxin to the root and inhibiting genes that negatively regulate the ethylene and jasmonic acid pathways, thereby promoting root growth and development (44). Elevated auxin levels in the root tip stimulate nitric oxide production, which supports the synthesis of strigolactones (SLs), thereby accelerating cell division. Strigolactones modulate root metabolic and developmental adaptations to low nitrogen availability, ensuring efficient uptake and translocation of available nitrogen. The accumulation of strigolactones promotes the degradation of D53 and releases GRF4, thus enhancing the expression of genes associated with nitrogen metabolism. Nitrogen limitation also induces the degradation of the DELLA protein SLENDER RICE 1 (SLR1) in a D14- and D53-dependent manner, releasing GRF4 from competitive inhibition by SLR1 (77). The phytohormone signaling in roots, such as IAA, CK, GA_3, JA, and ETH concentrations, is stimulated by low-nitrogen stress (56,78).

Genetic regulation

Nitrogen plays a vital role in plant growth and development, with roots playing a crucial role in enhancing nitrogen use efficiency and facilitating root development by regulating genes involved in nitrogen uptake and utilization, carbon metabolism, root growth, and the regulation of phytohormones. These adaptations help plants cope with low nitrogen conditions. When nitrogen levels are insufficient, roots elongate as a result of metabolic shifts induced by a sequence of gene expression changes. Several genes exhibit altered expression, playing a critical role in the plant's ability to adapt to low-nitrogen stress. Table 1 provides a list of important genes and transcription factors expressed under low nitrogen conditions.

Table 1. Expression of genes in cereals and transcription factors under low nitrogen conditions

Crop	Genes & transcription factors	Role	References
Rice	OsNRT1.1a	Enhanced plant height, biomass, yield, and nitrogen use efficiency	(90)
Rice	OsNRT2.5	Nitrate transport from roots to shoots	(91)
Rice	OsAMT1;2 and OsGOGAT1	Ammonium uptake and nitrogen remobilization at the whole plant level	(92)
Rice	OsGS1	Accumulation of total nitrogen	(93)
Rice	OsPIN9	An auxin efflux carrier modulated auxin response and increased yield	(94)
Barley	AlaAT	Improved nitrogen use efficiency	(95)
Wheat	TaGS1c, TaAlaAT, and TaPPDK	Enhanced nitrogen remobilization efficiency	(96)
Barley	HvNRT2.4, HvNRT2.1, and HvNRT2.2	Nitrate absorption and distribution	(74)
Wheat	Triticum aestivum Dof1 (TaDof1)	Nitrogen assimilation	(97)
Maize	GLK5, GLK8, NLP15 and bZIP108	GLK5, GLK8, and NLP15 - regulated genes suppressed under low-nitrogen conditions; bZIP108 - gene activation	(98)
Maize	MYB36 and AP2-EREBP	Nitrogen stress tolerance	(80)
Maize	ZmNLP5	Transcriptional regulation in the plant's response to low nitrogen stress	(82)

A genome-wide association study (GWAS) of two root morphological traits, namely root length and root diameter, identified six genes that were differentially expressed in a low-nitrogen tolerant variety. These six genes (*Os07g0471300, Os11g0230400, Os11g0229300, Os11g0229400*, *Os11g0618300,* and *Os11g0229333*) were identified as promising candidate genes for low-nitrogen tolerance (79).

The uptake and utilization of nitrate is a complex process that involves absorption, translocation, and assimilation. Several essential genes related to nitrogen uptake, assimilation, and metabolism, such as glutamine synthetase and asparagine synthetase, as well as genes involved in redox homeostasis like superoxide dismutase and peroxidase, were highly expressed under low nitrogen conditions (80). In addition to previously identified nitrogen response genes, such as nitrate transporter, nitrite reductase, nitrate reductase, and ferredoxin, new nitrogen response genes have been identified, including early light-inducible protein, uroporphyrinogen methyltransferase, phosphoenolpyruvate carboxylase, tonoplast intrinsic protein, and sesquiterpene cyclase (81). The up-regulation of *Exp* and *Nrt* gene family members has been shown to increase nitrogen absorption in cereal crops under low nitrogen (25). The genes related to the tricarboxylic acid and nicotinamide metabolic process (82) were also highly expressed. Furthermore, genes involved in sugar accumulation in the cell wall and enhanced cell wall synthesis, leading to increased thousand-grain weight, were expressed under low-nitrogen conditions (27).

The integration of genetic engineering and biotechnological tools into breeding programs is crucial for targeting specific candidate genes linked to enhance NUE. Molecular breeding approaches, such as QTL mapping, candidate gene identification, and marker-assisted selection (MAS), combined with advanced breeding strategies, can aid in development of genotypes with improved nitrogen recovery. Gene-editing technologies, such as CRISPR-Cas9, have revolutionized the creation of genetically engineered plants, enabling the development of high-yielding cultivars with superior NUE. Several nitrogen transporter genes, including *NRT1.1b, NAR2.1, NRT2.3a,* and *AMT1.1*, have been genetically modified in rice to enhance NUE (83). The Growth-regulating-factor 4 (OsGRF4), a key transcriptional regulator of nitrogen metabolism genes, antagonizes the DELLA growth repressor. CRISPR/Cas9 editing has demonstrated that modifying the OsGRF4-DELLA interaction by increasing OsGRF4 levels significantly enhances both NUE and grain yield (84).

Another promising target, the *ARE1* gene, was identified in GWAS as a regulator of NUE, functioning as a suppressor of plastidic Fd-GOGAT in rice. Loss-of-function mutations in *ARE1* in rice, wheat, and barley have resulted in improved yields and higher NUE, making it a valuable candidate for genome editing. CRISPR/Cas9-mediated editing of *ARE1* in wheat and barley has shown significant enhancements in NUE (85). Thus, the shift from traditional, time-consuming breeding methods to modern, efficient tools such as omics-based approaches and CRISPR/Cas9 gene-editing technology is well-justified.

MicroRNAs (miRNAs) are known to play a crucial role in regulating NUE and are often associated with various transcription factors. Various miRNAs contribute to the tolerance to low nitrogen stress (82). The miRNA169 family,for example, is known to regulate genes involved in nitrogen transport. New members of the miR169 family have been identified, and these miRNAs respond to nitrogen-deficient conditions (86). In cereals, the regulation of NAC genes by miR164 is thought to play a role in maintaining nitrogen remobilization from leaves to seeds under low-nitrogen conditions. New potential miR169 species, such as miRC10 and miRC68 (87), and low nitrogen-responsive TaMIRs are involved in plant tolerance to low-N stress (88). Altered expression of miRNAs associated with plant growth and development has been observed in various plant species exposed to abiotic stress conditions, including drought, salinity, extreme temperatures, nutrient deficiencies, and heavy metal toxicity. These findings suggest that miRNAs could serve as potential targets for genetic modifications aimed at enhancing nutrient stress tolerance in crop plants. Transgenic plants with miRNA overexpression have demonstrated improved tolerance or increased sensitivity to various abiotic stresses compared to their wild-type counterparts (89).

Conclusion

Nitrogen use efficiency in plants is a complex process influenced by various internal and external factors, including soil nitrogen availability, its uptake, and incorporation into biochemical compounds. This review highlights the morphological, physiological, biochemical, hormonal and genetic responses observed in cereals under low-nitrogen conditions. Such responses include root architecture modifications to enhance nitrogen acquisition, changes in photosynthetic efficiency, activation of nitrogen assimilation pathways, and genetic variations that promote NUE. Collectively, these traits provide a robust framework for identifying and selecting nitrogen-efficient genotypes. When incorporated into breeding programs, such traits can facilitate the development of nitrogen-efficient cereal varieties capable of sustaining high yields while reducing nitrogen fertilizer inputs.

Despite substantial progress in NUE research, several challenges remain, including complex trait interactions, environmental variability, limited marker utility, a lack of standardized phenotyping protocols, and underutilization of genome editing technologies. These challenges hinder the effectiveness of crop improvement programs targeting NUE in crops. Future research should prioritize the development of nitrogen-efficient cereal varieties with enhanced tolerance to low-nitrogen environments to address these issues. Advancing this goal necessitates the identification of key genes involved in nitrogen uptake, assimilation, and remobilization through approaches like genome-wide association studies (GWAS) and cutting-edge tools like CRISPR-Cas9 and genomic selection.

Achieving significant advancements in NUE requires a holistic approach that integrates plant breeding, advanced biotechnological innovations, high-throughput phenotyping techniques, agroecological practices, and supportive policy frameworks. Such efforts are essential to ensure sustainable agricultural practices and meet the growing demand for global food production.

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Authors' contributions

SD prepared the first draft of manuscript. SA, AK, DM, RV, PR and MS participated in editing and correcting the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: The author(s) declared that they have no competing interests.

Ethical issues: None

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