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A critical review of nitrogen metabolism and its role in improving cotton productivity

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ABSTRACT

Nitrogen is a vital macronutrient required for plant growth and development due to its essential role in amino acids, nucleic acids, chlorophyll, and various metabolic cofactors. Although nitrogen is the most abundant atmospheric element (78%), plants can utilize it only in ammonium (NH_4^+) and nitrate (NO_3^-) forms. While some nitrogen-fixing microbes help convert atmospheric nitrogen into plant-available forms, modern crop production mainly relies on inorganic fertilizers to meet nitrogen needs. In plants, nitrogen assimilation is tightly regulated by a coordinated enzyme network, including nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthase. In cotton, nitrogen availability directly influences vegetative–reproductive balance, chlorophyll content, boll formation, lint yield, and fiber quality. Therefore, improving nitrogen use efficiency (NUE) is critical for reducing production costs and increasing profitability. NUE can be enhanced through agronomic adjustments, physiological improvements, biological interventions, and genetic approaches aimed at strengthening innate nitrogen utilization pathways. Developing cotton cultivars with superior NUE will also contribute to environmental sustainability by reducing nitrogen losses through leaching and volatilization.

Keywords: Ammonium, Cotton, Nitrate, Nitrite, NUE, Reductases.

INTRODUCTION: All the biogeochemical cycles involve nitrogen element. Nitrogen also holds the fundamental position in all life forms due to its being part of amino acids and nucleic acid. Nitrogen mostly work as part of many chemical forms to facilitate its movement through atmosphere, lithosphere, hydrosphere and biosphere. Nitrogen keep on circulating on earth via nitrogen fixation, ammonification, nitrification and denitrification. Microorganisms, mostly bacteria are responsible for nitrogen fixation by converting atmospheric nitrogen into ammonia (NH_3). After fixation, ammonia is oxidized in nitrite (NO_2^-) and nitrates (NO_3^-) in the process of nitrification to make nitrogen available to the plants for absorption (Monib *et al.*, 2024). When nitrogen completes its work in plants and living organisms, denitrification occurs via bacterial activity to convert nitrates to gaseous nitrogen (figure 1).

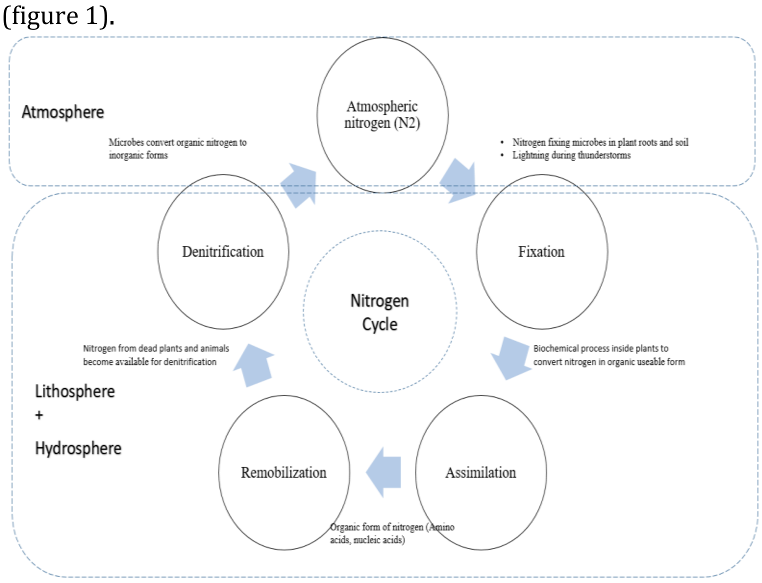

Figure 1: 1nitrogen cycle in atmosphere, hydrosphere and lithosphere.

Crop plants uptake nitrogen from soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-). This activity is mediated by complex transport system depending on the type of crop plants. In conditions where nutrients are poorly available, high affinity transport systems get activated (Akhtar *et al.*, 2024). Some legume crops uses symbiotic bacteria in low nitrogen available soils to fulfill nitrogen need of crop plants (Hestrin *et al.*, 2019; Perkowski *et al.*, 2024). These microbial communities in the soil are very crucial in the conversion of nitrogen. Such communities do not only channel the transformation of atmospheric nitrogen into forms consumed by plants but also play a role in the process such as mineralizing organic nitrogen and affecting the availability and uptake of nitrogen by plants (Koch and Sessitsch, 2024). Opportunistic mycorrhizal fungi have been shown to be very effective in terms of their capacity to acquire nitrogen especially in low-nitrogen environments (Hestrin *et al.*, 2019). However, the nitrogen cycle has been significantly changed due to human activities, with synthetic nitrogen fertilizers being one of them. The percentage of applied fertilizers wastage into the environment also leads to eutrophication of water bodies and emissions of nitrogenous gases that cause climate change (Monib *et al.*, 2024). Learning how the nitrogen uptake process occurs at the molecular level and the genetic networks by which they are controlled have opened the door to novel crop management methods and genetic engineering. The improvements in nitrogen utilization efficiency (NUE) by breeding or genetic modification of plants can be used to alleviate the impact on the environmental cost and improve crop productivity in changing environments (Kiba and Krapp, 2016). The nitrogen cycle is a complex cycle that guarantees the unending supply of this nutrient that is very vital among the ecosystems. The developments in the mechanics between plants and nitrogen-cycling micro-organisms have provided feasible solutions to improve ways of using nitrogen for sustainable agriculture production in the face of the environmental changes.

The objectives of this review are to examine the fundamental role of nitrogen and its dynamic behavior within cotton crop plants, to identify the various agronomic, physiological, biological, and genetic approaches that can enhance nitrogen use efficiency, and to highlight the existing limitations and challenges that hinder the improvement of nitrogen use efficiency in cotton production systems

Nitrogen in cotton: Nitrogen is an essential nutrient for growth and development of cotton crop plants alongside carbon, hydrogen, oxygen, phosphorus and potassium. It is one of the primary elements of the nucleic acid, amino acid, chlorophyll and enzymes. Especially in cotton, nitrogen supports canopy development, photosynthetic efficiency, and its sufficient availability is critical at boll development stage (Chen *et al.*, 2019; Shah *et al.*, 2022). Nitrogen deficiency in cotton plant causes leaf area reduction, lower photosynthetic rate, fewer bolls due to boll shedding and ultimately reduced lint yield (Wei *et al.*, 2024). Excess of nitrogen in soils would cause more vegetative growth, delayed maturity, less retention, poorer fiber quality (Chen *et al.*, 2019). Indeterminate growth habit of cotton and high mobility of nitrogen with in plants makes nitrogen management a difficult task. Thus, nitrogen fertilizer timing, amount and soil moisture must be considered carefully for fertilizer application. The inorganic nitrogen fertilizers are applied for better crop growth and yield but seepage of nitrates into the ground water and emission of nitrous oxide in atmosphere work as greenhouse gas (Khan *et al.*, 2017). An effective way of managing nitrogen in cotton is improving nitrogen use efficiency (NUE) of cultivars. It can be done with non-conventional fertilizers like Polymer-coated urea which is slow-release fertilizer, it will contribute to the increase of the yield and a reduction of the environmental pollution (Khan *et al.*, 2017). The fertilizer aid in upgrading physiological properties of cotton. Adequate level of nitrogen not only stimulates processes of photosynthesis but also stomata functioning which results in the increased biomass and improved growth performances (Chen *et al.*, 2019). The nitrogen levels in cotton plant is critical in determining the fertilizer doses that can be used by plants more efficiently. The nitrogen levels in plants and soils are now easier to determine by using sophisticated machine learning models to improve the nitrogen management practices in crop husbandry (Chen *et al.*, 2024). The fertilizer rate should be determined after considering different field factors including plant to plant distance depending on planting systems and application method to influence productivity and quality of fiber positively (Zaman *et al.*, 2021). Nitrogen availability can also be improved by crop rotation system involving legume which improve the provision of nitrogen in the soil. Especially, Faba beans can work in acidic soils and stores vast levels of nitrogen in soil that can be used later by cotton seedling more efficiently. This type of naturally available nitrogen facilitate in sustaining soil ecology and limiting the use of inorganic fertilizer (Li *et al.*, 2011). Other soil amendments like biochar usage can further improve soil conditions for better crop yields due to enhanced nitrogen fixation and soil fertility (Yadav *et al.*, 2017). Nitrogen management in cotton is a complicated issue that is currently managed by applying inorganic fertilizers, managing interplant distance, crop rotation and using artificial intelligence in determining crop nitrogen need and fulfilling accordingly. All these practices help in boosting sustainable high quality cotton lint production and minimizing environmental damage (Khan *et al.*, 2017; Zaman *et al.*, 2021). Optimum nitrogen levels in plants typically results in improved canopy photosynthesis, leaf weight and Crop yield (Wang *et al.*, 2012). After being absorbed by cotton plants in the form of nitrate (NO_3^-) and ammonia (NH_4^+), nitrogen gets assimilated in roots into organic compounds. Later on genetic regulations decides the nitrogen use efficiency of plants (Ninkuu *et al.*, 2023). Plant uses the assimilated organic nitrogen in formation of many Biomolecules. This process is highly dependent on the optimal levels of irrigation (Kumar *et al.*, 2022). Besides, plant spacing, coupled with the best nitrogen application, ensures increase the yield and fiber quality (Zaman *et al.*, 2021). Nitrogen does not fix in biomolecules, rather, it gets recycled in cotton plant via decomposition of organic substances and reallocation of nutrients to developing pats but the relocation is affected by environmental conditions like salinity (figure 2). Salinity stress not only hinder recycling of nitrogen but it also affect the rate of nitrogen absorption by roots, transport and also worsen soil nitrogen pollution (Heng *et al.*, 2024).

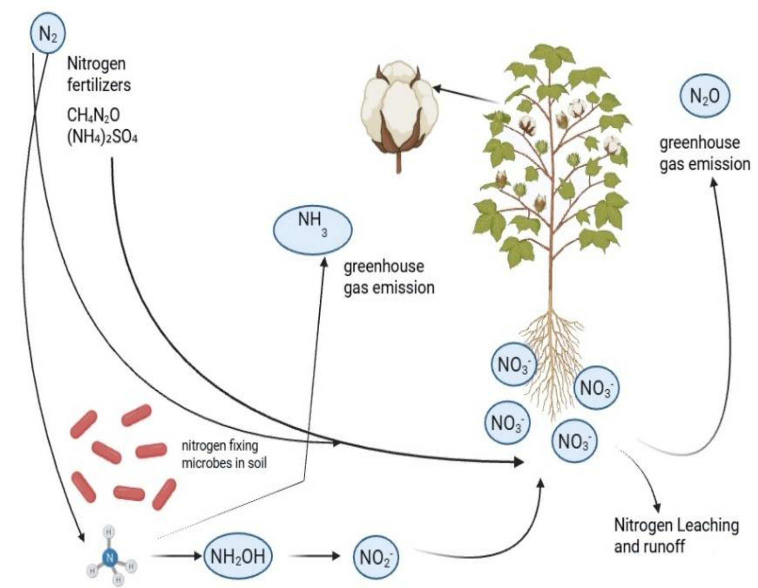

Figure 2: Nitrogen dynamics in cotton.

Biological nitrogen fixation: Nitrogen fixation involves the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). It is an evolutionary symbiotic process of delivering nitrogen by microbes to crop plants. The nitrogen fixation is not an innate process of cotton plants but scientists are working on stimulating symbiosis nitrogen fixation in non-leguminous crops, similar to symbiosis in legumes with *Rhizobium* spp. Going a step ahead of bacterial symbiosis, mycorrhizal fungus offers symbiosis that not only help plants survive in water deficit but also provide improved nutrient absorption. Arbuscular mycorrhiza shows affinity of symbiotic relation with cotton to modulate inorganic nitrogen assimilation more efficiently and also shows improved resistance to *verticillium* wilt (Zhang *et al.*, 2025). This type of symbiotic relation offers an alternative to synthetic fertilizers and can reduce nitrate pollution. It can be done either through direct symbiosis of microbes in roots of non-legume plans or via generally increasing the biological pool of nitrogen in the soil. Controlling the interactions of plant-rhizobia and the microbial communities in the rhizosphere can lead to the right direction of biological nitrogen available to cotton plants. This strategy will not only benefit the crop but also improve soil heath by reducing synthetic fertilizer use.

Molecular insight of nitrogen dynamics: An insight in the molecular processes involved in nitrogen dynamics inside the plants is key in improving their nitrogen use efficiency (Ninkuu *et al.*, 2023). Nitrate transporter family especially NRT1 and NRT2 is mostly involved in nitrogen uptake and transport to different parts of plant (Pu *et al.*, 2023). While in clayey, damp soils with high organic compounds, nitrogen is absorbed and assimilated in the form of ammonia by ammonia transporter familiar especially GhATM1.3 (Sun *et al.*, 2019). NRT1 family member, NRT1.1 not only act as transporter but also as transpector to control uptake of nitrate in response to environmental nitrate fluctuation levels (Gojon *et al.*, 2011; Wang *et al.*, 2020). Phosphorylation controls the NRT1.1 activity and changes the transporter state from low to high affinity and in this way enhancing nitrate uptake in nutrient deficient soil conditions (Parker and Newstead, 2014). NRT2 family, operates through high-affinity transport systems (HATS) and is responsible for nitrogen acquisition in low nitrate soils. The regulation of this family is both at gene expression level and post transcription modification, representing the complex nitrate uptake mechanism (Xu *et al.*, 2024). Cotton plant is also capable of absorbing ammonia along with nitrates but a high amount of ammonia is toxic to plants. In these conditions cotton plants assimilate ammonia into glutamine by using glutamine synthase (GS). Another enzyme, glutamate dehydrogenase (GDH) is further used by plants to convert glutamate to 2-oxoglutarate and ammonium to be used in assimilation of nitrogen (Labboun *et al.*, 2009). The coordinated activity of both these enzymes detoxified ammonia and maintain nitrogen assimilation to balance homeostasis under changing environmental conditions. Genetic studies revealed that, a member of glutamine synthase family namely GhGLN1.5, can potentially be used in regulating symbiotic relation of arbuscular mycorrhiza with cotton to resist *verticillium* wilt. This symbiotic relation modulates inorganic nitrogen assimilation more efficiently (Zhang *et al.*, 2025).

Biochemical nitrogen assimilation: Nitrogen assimilation in plants is complex process, which involve several enzymes to make inorganic nitrogen a part of amino acids and other nitrogenous materials. The nitrates (NO_3^-) absorbed by plants are first converted into ammonium (NH_4^+) via an enzymatic process which involve nitrate reductase enzyme. Nitrate reductase then nitrite reductase further into ammonium (Zayed *et al.*, 2023). The nitrate derived and directly absorbed ammonium is transformed in glutamine by an enzyme glutamine synthase (GS). Next step of nitrogen assimilation is construction of amino acids. For nitrogen from absorption to the amino acid formation some enzymes play critical role. These include glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AspAT) and asparagine synthetase (AS) (Kishorekumar *et al.*, 2019). However, the efficiency of nitrogen assimilation in plants is highly affected by environment including light intensity, soil pH, and soil health (Mokhele *et al.*, 2012). Studies have shown that carbon metabolism and nitrogen assimilation in plants are associated with each other. The carbon metabolism gets more important when plants absorb ammonium instead of nitrates, which need more carbon skeletons in addition to glutamine synthase (GS) to detoxify and transform ammonium to amino acids (Vega-Mas *et al.*, 2019; Giordano *et al.*, 2021).

Nitrogen transport and distribution: After assimilation in amino acid and nucleic acids, nitrogen is transported via sophisticated process to aid the development and growth of crop plants. Nitrogen is involved in cell division and elongation to boost the plant growth and especially fiber quality in cotton (Van Der Sluijs, 2022). Due to its importance in plant growth and development nitrogen is often applied at critical growth stages of cotton crop in wright way and rate to get maximum crop yields (Ninkuu *et al.*, 2023). Nitrogen interacts with other factors like irrigation to facilitate physiological growth via improving leaf area index, dry matter accumulation and crop growth rate. All these traits are crucial and ensure optimum plant growth to get maximum crop yield (Kumar *et al.*, 2022). Cotton canopy photosynthesis and leaf weight is also highly influenced by nitrogen supply to the plants. Both of these factors positively contribute to high cotton seed yield potential. Thus, nitrogen application method, time and element source should be managed to get desired crop growth and fiber quality results in cotton (El-Waraky *et al.*, 2025). Xylem and phloem both are involved in nitrogen transport inside the cotton plant. Xylem mostly transport inorganic form of nitrogen like nitrate in the flux of transpiration

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from roots to the sink parts of plant (figure 3). While, phloem mostly transfer organic form of nitrogen like amino acids in bidirectional ways. Nitrogen is thus distributed in different parts of plant to continue the growth and development (Yao *et al.*, 2020; Zayed *et al.*, 2023). The flexible transport system of nitrogen ensures its sufficient amount reaches the part of plant where it is needed. The active exchange between two systems of nutrient transport allows nitrogen movement in plant depending on development stage of the plant and environmental conditions (Notaguchi and Okamoto, 2015; Liu *et al.*, 2025). Genetic regulations can be studied and manipulated to improve nitrogen use efficiency of plants.

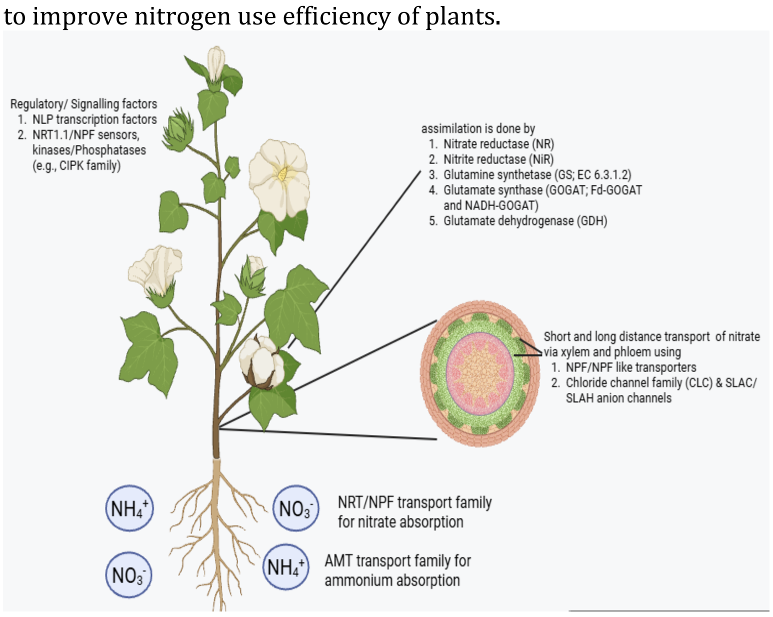

Figure 3: Nitrogen transport and distribution in cotton.

Nitrogen remobilization: Remobilization of nitrogen is vibrant physiological process of cotton plants which ensures nitrogen redistribution from older parts of plant to the new and actively developing parts. The nitrogen absorbed by cotton plants in early stages are assembled into nitrogenous compounds and stored mostly in vegetative parts of plants like leaves and stem. This stored nitrogen is the remobilized later by plant according to it need in developing and filling stage of bolls (Masclaux-Daubresse *et al.*, 2010). Plants has high nitrogen demand in boll and seed development stages, which is efficiently met with remobilization of nitrogen even in low nitrogen available soils (Liu *et al.*, 2021). About 70% of nitrogen in cotton seed is remobilized from vegetative tissues (Liu *et al.*, 2021). The efficiency of this physiological process highly depend on fertilizer application timing, plant genetic structure and environmental condition. Excess nitrogen application at late stages disrupt remobilization process and result in prolonged vegetative growth and low quality fiber (Wani *et al.*, 2021). Thus, considering remobilization dynamics is important in optimizing nitrogen use efficiency of crop plants and minimizing nitrogen losses to pollute environment.

Environment and nitrogen: The excess nitrogen applied to cotton crop causes imbalance in soil and excess nitrogen washes away in deeper soils and water bodies. This will end in greenhouse gasses including nitrous oxide, eutrophication and water contamination (Ahmed *et al.*, 2017; Tufail *et al.*, 2024). Water contamination and eutrophication cause hypoxic waterbodies that do not support any form of life. These dead areas are contaminating and effecting the whole aquatic biota (Giordano *et al.*, 2021). Additionally volatilization of ammonia from inorganic fertilizers and nitrous oxide gases emission leads the greenhouse gasses and ultimately are culprit of climate change (Ali *et al.*, 2025). The cultivars that have high nitrogen use efficiency no only decreases the cost of production but also facilitates the environmental stability in reduced greenhouse gas emission and ammonia volatilization. Thus, will ultimately limit the environment contamination (Shah *et al.*, 2022). However, only improving nitrogen use efficiency is not sufficient. Crop production must use efficient slow-release fertilizers instead of more damaging inorganic ones. The farming system on the whole must also be altered to used more manure-based fertilizers and explore symbiosis options for efficient nutrient availability. Both bacteria and fungus provide options to the symbiosis in cotton. These practices will not improve nitrogen in the soil but also improve soil structure and biota (Abd-Alla *et al.*, 2023).

Mechanized farming also provide efficient, timely and less environment damaging solution of fertilizer application for economically stable cotton production (Giordano *et al.*, 2021). Although, Bt. and GT GMO cotton has facilitated farmers to produce more quality yield of cotton seed but this genetic modification still depends on balanced fertilizers in soils to get stable yields. All in all, the introduction of Bt. and GT cotton has been advantageous to the global farmer with the creation of both economic and environmental benefits. The innovations demonstrate the possible role of genetically modified crops in improving agricultural efficiency and sustainability. This idea can be used to improve nitrogen use efficiency (Brookes, 2020).

Sustainable cotton production by improving NUE: About 150-300kg ha-1 nitrogen is used by cotton crop in one season depending on soil fertility, plant papulation, time of application and moisture contents of soil. One cotton plant uses 1-5 grams of nitrogen depending on plant papulation size (Chattha *et al.*, 2022). However, all the applied nitrogen does not gets available to the crop plants, instead it leaches down to the soil and water to damage environment and leading to the poor economic performance of cotton crop (Delate *et al.*, 2021; Wang *et al.*, 2021). The nitrogen use efficiency of cotton can be improved in several ways. Precision agriculture with altered fertilizer types that can slowly release nitrogen and getting the help of artificial intelligence to determine the time and amount of fertilizer are the immediate solution in this regard (Yadav *et al.*, 2017; Ali *et al.*, 2025). Crop rotation and exploration of symbiotic relations also offer affordable and practical ways to improve nitrogen use efficiency of cotton crop (Antille *et al.*, 2016; Wang *et al.*, 2021). However, the genetic manipulation of cotton offers the constant and sustainable solution to develop cultivars that will use nitrogen efficiently and will need a smaller number of fertilizers applications for getting higher crop yields. The more suitable solution will be using agronomic strategy and genetic manipulation all together to get sustainable cotton production (figure 4) and minimal environmental damage (Elrys *et al.*, 2022; Reddy *et al.*, 2025).

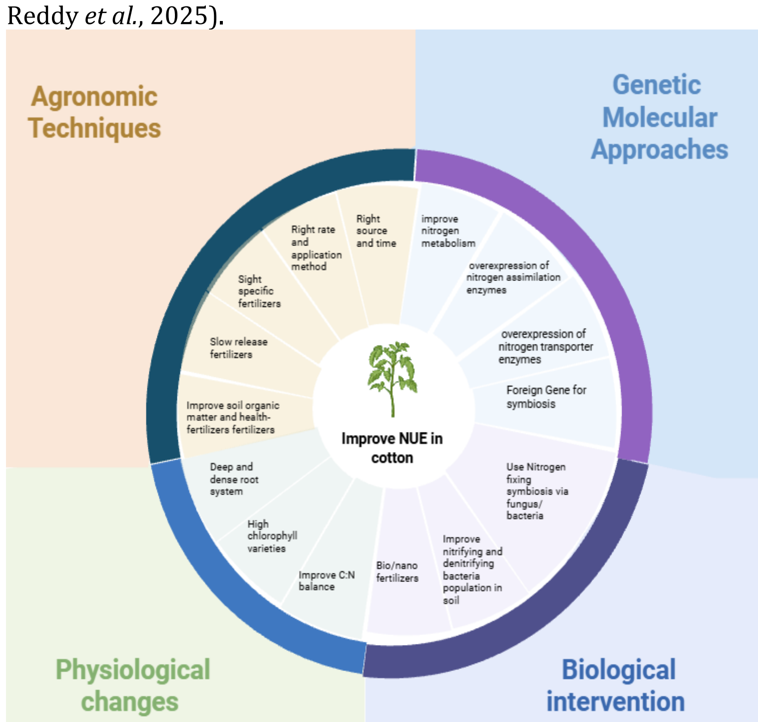

Figure 4: 4ways to improve nitrogen use efficiency in cotton

Genetic interventions: There are complex physiological and biochemical pathways which control nitrogen uptake and mobility in cotton plants. All these pathways are under precise genetic control and regulations. To improve nitrogen use efficiency in cotton plant genetic engineering can help in making more efficient biochemical and physiological pathways. It will improve nitrogen use efficiency and will ultimately lower the cost of fertilizer and environmental damage (Lebedev *et al.*, 2021; Ninkuu *et al.*, 2023). CRISPR/Cas9 offers accurate genome editing to improve nitrogen uptake and assimilation in cotton (Chen *et al.*, 2017). CRISPR/Cas9 has shown its potential in improving biomass, yield and offers sustainable solution of genetically improving nitrogen use efficiency. The regulatory processes of nitrogen relocation and assimilation can be comprehended and improve genetic modification (Karunarathne *et al.*, 2020; Karunarathne *et al.*, 2022). In general, using genetic engineering with modern agronomic methods is an opportunity to attain effective welfare of nitrogen usage in cotton. This will improve cotton production and help to achieve environmental sustainability through less nitrogen fertilizer that will be needed (Kumar *et al.*, 2022; Ali *et al.*, 2025).

Root architecture modification: Genetic engineering of cotton to enable better utilization of its root architecture, which allows ability of penetrating nitrogen-rich soils, is one way of better utilization of its nitrogen. This plan aims at altering genes related to creating the root and the development of nitrogen-absorbing structures that would go a long way in increasing the efficiency of the plant to avail nitrogen effectively thus lowering the reliance on fertilizers. Literature is strong on the fact that root biomass and architecture are important in uptake of nitrogen. As an example, it has been demonstrated that some root morphology adjustments, including root hair density and length, enhance the absorption of nitrogen in the situation of nitrogen deficiency (Zhu *et al.*, 2022). Also, biochar usage was reported to enhance the architecture of the root systems, as it leads to the enhancement of the efficiency of nitrogen assimilation (Feng *et al.*, 2021).

Moreover, genotypic differences have a strong effect, and certain genotypes are more efficient in nitrogen use (NUE) because they possess improved uptake and utilization tenets to nitrogen (Iqbal *et al.*, 2020). Improvement of NUE has also used molecular methods such as omics methods, which address both cellular and structural levels. With its emphasis, the sustainable increase in the NUE of cotton will be possible, which will positively affect the environment and agricultural output (Chattha *et al.*, 2022; Ninkuu *et al.*, 2023).

Nitrogen assimilation: The nitrogen which is absorbed by roots has to get assimilated into amino acids and nucleic acids via sophisticated assimilation pathways. These pathways include active participation of glutamine synthase (GS) and nitrate reductase (NR). The modification of genes encoding these enzymes can increase efficiency of nitrogen transformation and incorporation in proteins and other molecules (Pu *et al.*, 2025). The process of nitrate converting into nitrite is a vital process of nitrate assimilation because it is catalyzed by nitrate reductase (NR), which reduces nitrate to nitrite and is then reduced to ammonium and integrated into amino acids like glutamine under the catalytic action of glutamine synthase (GS). Higher concentration of nitrate reductase in crop plants have shown more efficient assimilation of nitrogen in organic compounds and ultimately improving nitrogen use efficiency of crop plants. Further, the amino acid formation by ammonium using glutamine synthase is important in making transportable nitrogen like glutamine and glutamate within plants (Ali, 2020; Zayed *et al.*, 2023). Thus genetic engineering for improved amount of glutamine synthase will also be helpful in utilizing nitrogen more efficiently. The genetic engineering of these pathways in other plant species has proven effective and there is a possibility to adapt the technology in cotton to improve its efficiency in utilizing nitrogen (Ninkuu *et al.*, 2023). As an example, the amplification of nitrate reductase activity and the regulation of glutamine synthetase activities in an organ or tissue specific manner have been shown to have constructive effects on the plant physiology and development under different nitrogen conditions. In addition, the use of such genetic enhances in breeding schemes might result into cotton species that do not need much nitrogen fertilizer, thus, lowering pollution rates to the environment and enhancing crop yield (Ali, 2020).

Symbiotic nitrogen fixation in cotton: Emulation of symbiotic fixation of nitrogen that occurs in leguminous crops such as soybean is one of the frontiers strategies of promoting nitrogen use efficiency (NUE) in crops including cotton. This can be done eider by direct inoculation of nitrogen fixing bacteria into the cotton root system or genetically engineering cotton to establish more efficient relationships with the bacterial plants. These types of methodologies can also help to achieve a high degree of less reliance on external nitrogen sources and thus promote sustainable agriculture. The symbiosis relation of nitrogen fixing rhizobia is an evolutionary success of these types of plants. To development of symbiosis in non-leguminous host plants like cotton comes with a number of complications (Oldroyd and Dixon, 2014; Mus *et al.*, 2016; Poole *et al.*, 2018). *Azorhizobium caulinodans* are diazotrophic microorganisms that do not need nodules and has potential to colonize some non-legume crops. They are able to proliferate intercellular spaces of the root system and can also enter into the internal system of the plant to form endophytic associations just like the nodular association observed in legumes (Kennedy *et al.*, 2005). Understanding the molecular activity that governs root nodule symbioses in legumes may lead to the creation of analogous associations in cotton and other crops that are not. To make such systems a reality in the field, it needs an interdisciplinary strategy, which means engaging synthetic biologists, microbiologists, plant biologists, breeders, agronomists, and even policymakers (Pankievicz *et al.*, 2019). The utilization of symbiotic nitrogen fixing processes in non-leguminous crops such as cotton is a promising and complex task, and it is necessary to address biological and genetic barriers to the creation and optimization of this association.

Genetic and molecular approaches: Cotton recycles the nitrogen in the senescent leaves to vigorously growing tissues across the period of development. This process is very important because it is the mechanism through which nitrogen is efficiently redistributed to other important sections of the plant thereby enhancing the overall growth and productivity of the plant. Genetic engineering provides potential possibilities of improving such a nitrogen remobilization. Multi-omics techniques proved its accuracy to determine the main genes and pathways in nitrogen metabolism, which can be exploited to increase NUE in cotton (Iqbal *et al.*, 2020). Moreover, posttranslational modification is also an important factor that constitutes the nitrogen use regulation and signaling, which implies that additional investigation into the modifications may bring new information on how to improve the use of the NUE at the molecular level (Wang *et al.*, 2021). Other solutions such as genetic modification of plants that have enhanced the rate of nitrogen metabolism have been considered to enhance the biomass and crop production (Lebedev *et al.*, 2021). In addition, the subsequent realization of the overall perspective on the genetic regulation of NUE in cotton might enhance the development of transgenic varieties with high nitrogen efficiency, which would ensure sustainable production of cotton (Ninkuu *et al.*, 2023). Thus, agronomic and molecular-based practices may be combined, which will provide a promising direction to improve the efficiency of nitrogen recycling in cotton and eventually make farming more efficient and sustainable (Chattha *et al.*, 2022). Not only does this development enable the growers to increase output and enhance the sustainability of the environment but also it is in line with the broader objectives of enhancing efficiency of resource utilization in the agricultural industry.

Improving NUE is only solution: The reliance on nitrogen fertilizers can be greatly minimized through improving the efficiency of nitrogen use efficiency (NUE) in cotton. Not only this reduces the negative effects on the environment like lower emission of greenhouse gases and lower forms of water contamination, but also helps create a more sustainable and environmentally friendly system of cotton production. Overuse of nitrogen fertilizers in cotton production usually results in low yield and pollution of the environment caused by the leaching of nitrates and release of nitrous oxide. Therefore, the enhancement of NUE opens the possibility of ensuring a more sustainable development by balancing the ratio of nitrogen supply and uptake by a plant (Yadav *et al.*, 2017; Shah *et al.*, 2022). Furthermore, the agronomic and molecular approaches involved to boost NUE has not only the outcome of improving nitrogen uptake of cotton plants but also addition to the yield per hectare of cotton. With such a combined method, it assists in enhancing cotton cultivar absorption and transformation functions as well as lowers the input expenses and boosts the profitability (Niu *et al.*, 2020; Chattha *et al.*, 2022). NUE could also be enhanced through the usage of the innovative agricultural methods, including but not limited to precision agriculture and the use of specific nutrient management (Reddy *et al.*, 2025). The ecological sphere gains the advantages of enhancing NUE because the ecological stressor in this area is considerably decreased due to the methods of fertilizers application. Better management strategies are to optimize nitrogen levels and use nitrogen-efficient cultivars to make sure that cotton production does not suffer or decrease but improves and also minimizes the negative effects on the environment (Scheer *et al.*, 2023). This multi-level approach, therefore, helps to sustain and reduce the cotton production systems in the long term (Li *et al.*, 2017).

Economic and agronomic benefits: In cotton farming, a large part of the expenditure on production goes in nitrogen fertilizers. Through the enhancement of nitrogen use efficiency (NUE) farmers will be in a position to minimize their reliance on expensive nitrogen inputs and as a result, it lowers production costs and increases profitability. Improvement of NUE in cotton can be achieved through different measures such as the improvement of the rate of application of nitrogen, the use of nitrogen efficient genotypes and adoption of new agronomic practices. It has been proven that NUE and cotton yield can be greatly increased by the use of integrated management practices, including the so-called 4R framework specifying right product, right rate, right time, and right place (Chattha *et al.*, 2022; Shah *et al.*, 2022). Moreover, cultivars capable of utilizing nitrogen efficiently and its prudent use and recycling are capable of increasing nitrogen uptake, its use, and remobilization, which eventually can increase the sustainability of cotton production ((Iqbal *et al.*, 2020; Mahboob *et al.*, 2024). Thus, by targeting NUE one will be able to reduce the environmental impact and make cotton production sustainable, providing significant economic gains to farmers (Reddy *et al.*, 2025)

Cotton growth, yield, and fiber quality: Cotton growth and yield are remarkably promoted by the effective utilization of nitrogen, which is mainly ideal in terms of nitrogen use efficiency (NUE). Enhanced NUE will see to it that there is more nitrogen to be used by other essential cellular functions as well as by plants to develop, which yields improved productivity despite less fertilization. This method does not only aid in sustainable agriculture, but also keeps the quality of fiber at a steady level. Studies have shown that NUE can be significantly enhanced through some optimization of nitrogen rate, source, timing and method of application. Genetic and molecular innovation can as well be valuable in achieving optimal nitrogen uptake and its proper utilization, which will eventually increase the agricultural production and profitability of cotton (Niu *et al.*, 2020; Shah *et al.*, 2022).

Climate change adaptation strategy in cotton: With the growing threat of climate change affecting the world cotton production through rising temperatures, new precipitation patterns, and the occurrence of extreme weather events (Ahmad *et al.*, 2023; Chatterjee *et al.*, 2023) in the food sector, improving nitrogen use efficiency (NUE) nowadays turns out to be a most important adaptation approach towards sustaining agricultural output under the new environment (Govindasamy *et al.*, 2023). The climate model forecasts 35-60% of decrease in cotton yield in mid-century under the high-emission conditions mainly due to heat and drought stress in the semi-arid areas (Ahmad *et al.*, 2023). At the same time, the increase in the temperature changes the dynamics of the nitrogen cycle and the absorption of nutrients by crops, which produces disproportionate impacts in different zones and makes controlling the results of fertilizers more difficult (Chatterjee *et al.*, 2023). Improved resilience of cotton crop by enhancing nitrogen use efficiency can help a great deal by several mechanisms. Nitrogen efficiency conserves fundamental physiological processes such as photosynthesis, nitrogen resources during reproduction, antioxidant mechanisms to enable plants to withstand heat, drought, and waterlogging stress (Shah *et al.*, 2022). Accurate nitrogen systems (leaf color chart-based applications and site-specific timing) have shown their capability to keep the yields on par with the high-input systems and enhance the efficiency of nitrogen recovery and minimize environmental losses (Gupta *et al.*, 2022). Intermediate rates of nitrogen (190270 kg/ha) result in a maximum seed cotton yield and reproductive nitrogen accumulation and do not result in soil buildup, which has shown in long-term field trials to be optimal in nitrogen management to achieve short- and long-term productivity and sustainability (Ma *et al.*, 2023). According to meta-analytic evidence, which was conducted on 61 studies, the optimal ratios of nitrogen to optimal rates were found to enhance the efficiency of nitrogen use by 44.6% and the seed cotton yield by 16%, underscoring the high value of calibrated nitrogen rates (Wang *et al.*, 2024). Improved-efficiency fertilizer such as slow-release formulations and controlled-release formulations also lead to improved plant nitrogen uptake and less volatilization and leaching loss, which permitted cotton crops to do better in unpredictable water regimes and with scarce fertilizer (Tartaglia *et al.*, 2020; Shah *et al.*, 2022). Their benefits are especially important to those areas with low nitrogen content and in times of fertilizer shortage, as they ensure the optimal growth of current amounts of nitrogen with the least losses of nutrients to the environment (Tartaglia *et al.*, 2020; Govindasamy *et al.*, 2023). Combination of agronomic, technological, and molecular solutions to nitrogen use performance is a holistic approach to climate change adaptation in cotton production systems, providing opportunities to sustain stable outputs despite the growing environmental limitations and resource shortages (Tartaglia *et al.*, 2020; Shah *et al.*, 2022; Govindasamy *et al.*, 2023).

Challenges and regulatory barriers: Although promising, there have been many challenges related to the discovery of genetically modified (GM) cotton containing a superior nitrogen use efficiency (NUE). A key challenge is related to the complexity of the nitrogen metabolism that incorporates a great number of genes and pathways. It is of great importance to have a complete knowledge of these processes to ensure that the desired improvements are attained without much undesirable side effects. Some of the most important processes in the metabolism of nitrogen are nitrogen uptake, assimilation, and remobilization, which interdepend with one another in a complex manner, which contributes to rendering this a challenging target of genetic modification (Lebedev *et al.*, 2021). Besides that, genetic modifications need to be tested on different types of soils, climate, and types of cotton to guarantee their high applicability and competence. Changes in environmental circumstances may have a substantial effect on the performance of genetically modified crops, and there is a tendency to assess their functionality in many different environmental conditions (Reddy *et al.*, 2025). What is more, the regulations of genetically modified organisms (GMOs) are different in different places, another drop of spice on the rose. Non-harmonized and prescriptive regulations that cannot solely influence the acceptance and commercialization of GM cotton with improved NUE, and the perception and knowledge of the population concerning GM technology may impact the problem. Overcoming all of these difficulties and securing the further progress of GM crops development will be impossible without ensuring proper communication patterns and optimal regulatory measures (Huesing *et al.*, 2016). The following challenges underscore the importance of the continuous research and the interdisciplinary cooperation to enhance NUE in cotton and other crops by combining genetic opportunities and agronomical techniques to benefit from sustainable crop production (Ali *et al.*, 2025). Among the recurrent obstacles to the development and adoption of genetically modified organisms (GMOs) is the regulatory and societal perception obstacle that has slowed and hindered the progress of GMOs. Even with the provable environmental and economic solution of increased Nitrogen Use Efficiency (NUE) in cotton, opposition to genetically modified (GM) crops remains as a major barrier to the practices (Smyth, 2017). Misinformation and lack of knowledge regarding GMOs often encourages the opposition of the population, causing delays in regulations and issues in international trade, especially in such areas as Europe (Dolezel *et al.*, 2024). The improved NUE of cotton is the illustration of GM crops to decrease harmful impacts on the environment as well as elevate productivity and efficiency of agricultural practices. GM cotton will have the potential to increase yields and cut down the proportion of fertilizer consumption by improving the absorption and efficiency of nitrogen, and this, in turn, has a substantial environmental impact, such as a decrease in nitrate pollution and increases in farm sustainability (Chattha *et al.*, 2022; Shah *et al.*, 2022). Nonetheless, the benefits notwithstanding, regulatory regimes especially across Europe are harsh in their demands on GMOs. Such legal obstacles are usually due to a robust anti-GMO movement, which influences the minds of the population and makes the policy implementation more complex (Landrigan and Benbrook, 2015; Catherine *et al.*, 2024). These challenges can be improved with long-term work in the field of education and open dialogue on the issue of GM cotton safety and sustainability. Even the scientific consensus about the safety of GMOs is in most cases not enough to change the way people perceive them because they are deeply rooted in the already formed attitudes concerning biotechnology (Landrum *et al.*, 2019).

CONCLUSION: Nitrogen is a critical nutrient for cotton growth, yield, and fiber quality, and its efficient management is essential for sustainable production. Improving nitrogen use efficiency (NUE) through optimized fertilization, crop rotation, biochar application, and mechanized precision agriculture can enhance productivity while minimizing environmental pollution. Advances in molecular biology and genetic engineering, including CRISPR/Cas9 and manipulation of nitrogen transporters and assimilation enzymes, offer promising avenues to develop cotton cultivars with superior NUE. Symbiotic interactions with nitrogen-fixing microbes and mycorrhizal fungi further provide sustainable alternatives to synthetic fertilizers. Despite challenges such as environmental variability, regulatory barriers, and societal perceptions of GM crops, integrating agronomic, molecular, and biotechnological approaches can ensure high cotton yields, environmental protection, and economic benefits. Enhancing NUE in cotton is therefore a key strategy for achieving resilient and sustainable cotton production under changing climate conditions

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REFERENCES: Abd-Alla, M. H., S. M. Al-Amri and A.-W. E. El-Enany, 2023. Enhancing rhizobium–legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. Agriculture, 13(11): 2092.

Ahmad, S., I. Ahmad, B. Ahmad, A. Ahmad, A. Wajid, T. Khaliq, G. Abbas, C. J. Wilkerson and G. Hoogenboom, 2023. Regional integrated assessment of climate change impact on cotton production in a semi-arid environment. Climate Research, 89: 113-132.

Ahmed, M., M. Rauf, Z. Mukhtar and N. A. Saeed, 2017. Excessive use of nitrogenous fertilizers: An unawareness causing serious threats to environment and human health. Environmental Science and Pollution Research, 24(35): 26983-26987.

Akhtar, K., N. U. Ain, P. V. Prasad, M. Naz, M. M. Aslam, I. Djalovic, M. Riaz, S. Ahmad, R. K. Varshney and B. He, 2024. Physiological, molecular, and environmental insights into plant nitrogen uptake, and metabolism under abiotic stresses. The plant genome, 17(2): e20461.

Ali, A., 2020. Nitrate assimilation pathway in higher plants: Critical role in nitrogen signalling and utilization. Plant Sci. Today, 7(2): 182-192.

Ali, A., N. Jabeen, R. Farruhbek, Z. Chachar, A. A. Laghari, S. Chachar, N. Ahmed, S. Ahmed and Z. Yang, 2025. Enhancing nitrogen use efficiency in agriculture by integrating agronomic practices and genetic advances. Frontiers in Plant Science, 16: 1543714.

Antille, D. L., J. M. Bennett and T. A. Jensen, 2016. Soil compaction and controlled traffic considerations in australian cotton-farming systems. Crop and Pasture Science, 67(1): 1-28.

Brookes, G., 2020. Genetically modified (gm) crop use in colombia: Farm level economic and environmental contributions. GM Crops & Food, 11(3): 140-153.

Catherine, K. N., B. R. Mugiira and N. J. Muchiri, 2024. Public perception of genetically modified organisms and the implementation of biosafety measures in kenya. Advances in Agriculture, 2024(1): 5544617.

Chatterjee, D., S. Saha, T. Bera and D. Saha, 2023. Mitigation and adaptation strategies involving nitrogen management for enhancing agro-ecosystem productivity and resilience to future climate change. Frontiers Media SA: pp: 1273821.

Chattha, M. S., Q. Ali, M. Haroon, M. J. Afzal, T. Javed, S. Hussain, T. Mahmood, M. K. Solanki, A. Umar and W. Abbas, 2022. Enhancement of nitrogen use efficiency through agronomic and molecular based approaches in cotton. Frontiers in Plant Science, 13: 994306.

Chen, J., L. Liu, Z. Wang, H. Sun, Y. Zhang, Z. Bai, S. Song, Z. Lu and C. Li, 2019. Nitrogen fertilization effects on physiology of the cotton boll–leaf system. Agronomy, 9(6): 271.

Chen, P., J. Dai, G. Zhang, W. Hou, Z. Mu and Y. Cao, 2024. Diagnosis of cotton nitrogen nutrient levels using ensemble mobilenetv2fc, resnet101fc, and densenet121fc. Agriculture, 14(4): 525.

Chen, X., X. Lu, N. Shu, S. Wang, J. Wang, D. Wang, L. Guo and W. Ye, 2017. Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the crispr/cas9 system. Scientific Reports, 7(1): 44304.

Delate, K., B. Heller and J. Shade, 2021. Organic cotton production may alleviate the environmental impacts of intensive conventional cotton production. Renewable Agriculture and Food Systems, 36(4): 405-412.

Dolezel, M., A. Lang, A. Greiter, M. Miklau, M. Eckerstorfer, A. Heissenberger, E. Willée and W. Züghart, 2024. Challenges for the post-market environmental monitoring in the european union imposed by novel applications of genetically modified and genome-edited organisms. BioTech, 13(2): 14.

El-Waraky, E. A., H. S. El-Beltagi, M. F. El-Nady, M. I. Al-daej, E. B. Belal, W. F. Shehata, M. L. Hadid and M. M. Salem Metwaly, 2025. Responses of gossypium barbadense l. Cotton plants to biofertilizers under different levels of nitrogen fertilization. Polish Journal of Environmental Studies, 34(1).

Elrys, A. S., A. S. Elnahal, A. I. Abdo, E.-S. M. Desoky, E. Selem and M. M. Rady, 2022. Traditional, modern, and molecular strategies for improving the efficiency of nitrogen use in crops for sustainable agriculture: A fresh look at an old issue. Journal of Soil Science and Plant Nutrition, 22(3): 3130-3156.

Feng, L., W. Xu, G. Tang, M. Gu and Z. Geng, 2021. Biochar induced improvement in root system architecture enhances nutrient assimilation by cotton plant seedlings. BMC Plant Biology, 21(1): 269.

Giordano, M., S. A. Petropoulos and Y. Rouphael, 2021. The fate of nitrogen from soil to plants: Influence of agricultural practices in modern agriculture. Agriculture, 11(10): 944.

Gojon, A., G. Krouk, F. Perrine-Walker and E. Laugier, 2011. Nitrate transceptor (s) in plants. Journal of experimental botany, 62(7): 2299-2308.

Govindasamy, P., S. K. Muthusamy, M. Bagavathiannan, J. Mowrer, P. T. K. Jagannadham, A. Maity, H. M. Halli, S. GK, R. Vadivel and D. TK, 2023. Nitrogen use efficiency—a key to enhance crop productivity under a changing climate. Frontiers in Plant Science, 14: 1121073.

Gupta, R. K., A. Shankar, R. Bhatt, A. A. Al-Huqail, M. H. Siddiqui and R. Kumar, 2022. Precision nitrogen management in bt cotton (gossypium hirsutum) improves seed cotton yield and nitrogen use efficiency, and reduces nitrous oxide emissions. Sustainability, 14(4): 2007.

Heng, T., Y. Ma, P. Ai, Z. Liu, M. Wu and C. Liu, 2024. The effects of soil salt stress on the nitrogen uptake, yield response and nitrogen use efficiency of cotton in arid areas. Agronomy, 14(1): 229.

Hestrin, R., E. C. Hammer, C. W. Mueller and J. Lehmann, 2019. Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. Communications biology, 2(1): 233.

Huesing, J. E., D. Andres, M. P. Braverman, A. Burns, A. S. Felsot, G. G. Harrigan, R. L. Hellmich, A. Reynolds, A. M. Shelton and W. Jansen van Rijssen, 2016. Global adoption of genetically modified (gm) crops: Challenges for the public sector. Journal of agricultural and food chemistry, 64(2): 394-402.

Iqbal, A., Q. Dong, X. Wang, H. Gui, H. Zhang, X. Zhang and M. Song, 2020. Transcriptome analysis reveals differences in key genes and pathways regulating carbon and nitrogen metabolism in cotton genotypes under n starvation and resupply. International journal of molecular sciences, 21(4): 1500.

Karunarathne, S. D., Y. Han, X.-Q. Zhang and C. Li, 2020. Advances in understanding the molecular mechanisms and potential genetic improvement for nitrogen use efficiency in barley. Agronomy, 10(5): 662.

Karunarathne, S. D., Y. Han, X. Q. Zhang and C. Li, 2022. Crispr/cas9 gene editing and natural variation analysis demonstrate the potential for hvare1 in improvement of nitrogen use efficiency in barley. Journal of integrative plant biology, 64(3): 756-770.

Kennedy, I. R., A. Choudhury, M. L. Kecskés, R. J. Roughley and N. T. Hien, 2005. Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited? In: Biological nitrogen fixation, sustainable agriculture and the environment: proceedings of the 14th international nitrogen fixation congress. Springer: pp: 271-272.

Khan, A., D. K. Y. Tan, F. Munsif, M. Z. Afridi, F. Shah, F. Wei, S. Fahad and R. Zhou, 2017. Nitrogen nutrition in cotton and control strategies for greenhouse gas emissions: A review. Environmental Science and Pollution Research, 24(30): 23471-23487.

Kiba, T. and A. Krapp, 2016. Plant nitrogen acquisition under low availability: Regulation of uptake and root architecture. Plant and Cell Physiology, 57(4): 707-714.

Kishorekumar, R., M. Bulle, A. Wany and K. J. Gupta, 2019. An overview of important enzymes involved in nitrogen assimilation of plants. Nitrogen metabolism in plants: methods and protocols: 1-13.

Koch, H. and A. Sessitsch, 2024. The microbial-driven nitrogen cycle and its relevance for plant nutrition. Journal of experimental botany, 75(18): 5547-5556.

Kumar, R., N. K. Pareek, U. Kumar, T. Javed, A. A. Al-Huqail, V. S. Rathore, V. Nangia, A. Choudhary, G. Nanda and H. M. Ali, 2022. Coupling effects of nitrogen and irrigation levels on growth attributes, nitrogen use efficiency, and economics of cotton. Frontiers in Plant Science, 13: 890181.

Labboun, S., T. Tercé-Laforgue, A. Roscher, M. Bedu, F. M. Restivo, C. N. Velanis, D. S. Skopelitis, P. N. Moshou, K. A. Roubelakis-Angelakis and A. Suzuki, 2009. Resolving the role of plant glutamate dehydrogenase. I. In vivo real time nuclear magnetic resonance spectroscopy experiments. Plant and Cell Physiology, 50(10): 1761-1773.

Landrigan, P. J. and C. Benbrook, 2015. Gmos, herbicides, and public health. New England Journal of Medicine, 373(8): 693-695.

Landrum, A. R., W. K. Hallman and K. H. Jamieson, 2019. Examining the impact of expert voices: Communicating the scientific consensus on genetically-modified organisms. Environmental Communication, 13(1): 51-70.

Lebedev, V. G., A. A. Popova and K. A. Shestibratov, 2021. Genetic engineering and genome editing for improving nitrogen use efficiency in plants. Cells, 10(12): 3303.

Li, C.-J., Y.-Y. Li, C.-B. Yu, J.-H. Sun, P. Christie, M. An, F.-S. Zhang and L. Li, 2011. Crop nitrogen use and soil mineral nitrogen accumulation under different crop combinations and patterns of strip intercropping in northwest china. Plant and Soil, 342(1): 221-231.

Li, P., H. Dong, C. Zheng, M. Sun, A. Liu, G. Wang, S. Liu, S. Zhang, J. Chen and Y. Li, 2017. Optimizing nitrogen application rate and plant density for improving cotton yield and nitrogen use efficiency in the north china plain. Plos one, 12(10): e0185550.

Liu, H., X. Gao, W. Fan and X. Fu, 2025. Optimizing carbon and nitrogen metabolism in plants: From fundamental principles to practical applications. Journal of integrative plant biology.

Liu, Z., C. Hu, Y. Wang, Y. Sha, Z. Hao, F. Chen, L. Yuan and G. Mi, 2021. Nitrogen allocation and remobilization contributing to low-nitrogen tolerance in stay-green maize. Field Crops Research, 263: 108078.

Ma, Y., H. Sun, Y. Yang, Z. Li, P. Li, Y. Qiao, Y. Zhang, K. Zhang, Z. Bai and A. Li, 2023. Long-term nitrogen fertilizer management for enhancing use efficiency and sustainable cotton (*Gossypium hirsutum* L.). Frontiers in Plant Science, 14: 1271846.

Mahboob, W., N. Sarwar, O. Bin Abdul Hafeez, M. A. R. Arif, M. Akhtar and G. Yang, 2024. Exploring genotypic variations in cotton associated with growth and nitrogen use efficiency. Journal of Plant Nutrition, 47(19): 3231-3250.

Masclaux-Daubresse, C., F. Daniel-Vedele, J. Dechorgnat, F. Chardon, L. Gaufichon and A. Suzuki, 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Annals of botany, 105(7): 1141-1157.

Mokhele, B., X. Zhan, G. Yang and X. Zhang, 2012. Nitrogen assimilation in crop plants and its affecting factors. Canadian Journal of Plant Science, 92(3): 399-405.

Monib, A., P. Niazi, S. Barai, B. Sawicka, A. Baseer, A. Nikpay, S. Fahmawi, D. Singh, M. Alikhail and B. Thea, 2024. Nitrogen cycling dynamics: Investigating volatilization and its interplay with n2 fixation. Journal for Research in Applied Sciences and Biotechnology, 3(1): 17-31.

Mus, F., M. B. Crook, K. Garcia, A. Garcia Costas, B. A. Geddes, E. D. Kouri, P. Paramasivan, M.-H. Ryu, G. E. Oldroyd and P. S. Poole, 2016. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Applied and environmental microbiology, 82(13): 3698-3710.

Ninkuu, V., Z. Liu and X. Sun, 2023. Genetic regulation of nitrogen use efficiency in gossypium spp. Plant, Cell & Environment, 46(6): 1749-1773.

Niu, J., H. Gui, A. Iqbal, H. Zhang, Q. Dong, N. Pang, S. Wang, Z. Wang, X. Wang and G. Yang, 2020. N-use efficiency and yield of cotton (g. Hirsutumn l.) are improved through the combination of n-fertilizer reduction and n-efficient cultivar. Agronomy, 11(1): 55.

Notaguchi, M. and S. Okamoto, 2015. Dynamics of long-distance signaling via plant vascular tissues. Frontiers in Plant Science, 6: 161.

Oldroyd, G. E. and R. Dixon, 2014. Biotechnological solutions to the nitrogen problem. Current opinion in biotechnology, 26: 19-24.

Pankievicz, V. C., T. B. Irving, L. G. Maia and J.-M. Ané, 2019. Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops. BMC biology, 17(1): 99.

Parker, J. L. and S. Newstead, 2014. Molecular basis of nitrate uptake by the plant nitrate transporter nrt1. 1. Nature, 507(7490): 68-72.

Perkowski, E. A., J. Terrones, H. L. German and N. G. Smith, 2024. Symbiotic nitrogen fixation reduces belowground biomass carbon costs of nitrogen acquisition under low, but not high, nitrogen availability. AoB Plants, 16(5): plae051.

Poole, P., V. Ramachandran and J. Terpolilli, 2018. Rhizobia: From saprophytes to endosymbionts. Nature Reviews Microbiology, 16(5): 291-303.

Pu, Y., P. Wang, M. Abbas, A. Iqbal, Q. Dong, T. Luo, Q. Wang, F. Cao and M. Song, 2025. Insights from model plants to improve cotton's use of nitrogen and phosphorus. Journal of Cotton Research, 8(1): 16.

Pu, Y., P. Wang, M. Abbas, M. A. Khan, J. Xu, Y. Yang, T. Zhou, K. Zheng, Q. Chen and G. Sun, 2023. Genome-wide identification and analyses of cotton high-affinity nitrate transporter 2 family genes and their responses to stress. Frontiers in Plant Science, 14: 1170048.

Reddy, M. B., P. Sravani, S. Kumar, M. V. S. Rajawat, D. K. Jaiswal, S. Dhar, E. A. Azman, K. Garg and S. Kumar, 2025. Nitrogen use efficiency reimagined: Advancements in agronomic, ecophysiological, and molecular strategies. Journal of Plant Nutrition, 48(9): 1577-1603.

Scheer, C., D. W. Rowlings, D. L. Antille, M. De Antoni Migliorati, K. Fuchs and P. R. Grace, 2023. Improving nitrogen use efficiency in irrigated cotton production. Nutrient Cycling in Agroecosystems, 125(2): 95-106.

Shah, A. N., T. Javed, R. K. Singhal, R. Shabbir, D. Wang, S. Hussain, H. Anuragi, D. Jinger, H. Pandey and N. R. Abdelsalam, 2022. Nitrogen use efficiency in cotton: Challenges and opportunities against environmental constraints. Frontiers in Plant Science, 13: 970339.

Smyth, S. J., 2017. Genetically modified crops, regulatory delays, and international trade. Food and Energy Security, 6(2): 78-86.

Sun, Y. C., S. Sheng, T. F. Fan, L. Liu, J. Ke, D. B. Wang, J. P. Hua, L. H. Liu and F. Q. Cao, 2019. Molecular identification and functional characterization of ghamt1. 3 in ammonium transport with a high affinity from cotton (*Gossypium hirsutum* L.). Physiologia Plantarum, 167(2): 217-231.

Tartaglia, F. d. L., A. R. E. d. Souza, A. P. d. Santos, A. P. Barros Júnior, L. M. d. Silveira and M. G. d. Santos, 2020. Eficiência no uso de nitrogênio por cultivares de algodão naturalmente colorido em região semiárida. Revista Ciência Agronômica, 51: e20196642.

Tufail, M. A., M. Ayyub, L. Tariq, J. Iltaf, A. Asbat, I. Bashir and W. Umar, 2024. Nitrogen fertilizers and the future of sustainable agriculture: A deep dive into production, pollution, and mitigation measures. Soil Science and Plant Nutrition, 70(5-6): 457-477.

Van Der Sluijs, M. H., 2022. Effect of nitrogen application level on cotton fibre quality. Journal of Cotton Research, 5(1): 9.

Vega-Mas, I., C. Cukier, I. Coleto, C. Gonzalez-Murua, A. M. Limami, M. B. González-Moro and D. Marino, 2019. Isotopic labelling reveals the efficient adaptation of wheat root tca cycle flux modes to match carbon demand under ammonium nutrition. Scientific Reports, 9(1): 8925.

Wang, D., M. W. Maughan, J. Sun, X. Feng, F. Miguez, D. Lee and M. C. Dietze, 2012. Impact of nitrogen allocation on growth and photosynthesis of miscanthus (miscanthus× giganteus). Gcb Bioenergy, 4(6): 688-697.

Wang, W., B. Hu, A. Li and C. Chu, 2020. Nrt1. 1s in plants: Functions beyond nitrate transport. Journal of experimental botany, 71(15): 4373-4379.

Wang, W., A. Li, Z. Zhang and C. Chu, 2021. Posttranslational modifications: Regulation of nitrogen utilization and signaling. Plant and Cell Physiology, 62(4): 543-552.

Wang, Z., K. Zhang, G. Shao, J. Lu, Y. Gao and E. Song, 2024. Water and nitrogen use efficiencies in cotton production: A meta-analysis. Field Crops Research, 309: 109322.

Wani, S. H., R. Vijayan, M. Choudhary, A. Kumar, A. Zaid, V. Singh, P. Kumar and J. K. Yasin, 2021. Nitrogen use efficiency (nue): Elucidated mechanisms, mapped genes and gene networks in maize (*Zea mays* L.). Physiology and Molecular Biology of Plants, 27(12): 2875-2891.

Wei, K., Q. Wang, M. Deng, S. Lin and Y. Guo, 2024. Response of cotton growth, yield, and water and nitrogen use efficiency to nitrogen application rate and ionized brackish water irrigation under film-mulched drip fertigation. Frontiers in Plant Science, 15: 1361202.

Xu, N., L. Cheng, Y. Kong, G. Chen, L. Zhao and F. Liu, 2024. Functional analyses of the nrt2 family of nitrate transporters in arabidopsis. Frontiers in Plant Science, 15: 1351998.

Yadav, M., R. Kumar, C. Parihar, R. Yadav, S. Jat, H. Ram, R. Meena, M. Singh, A. Verma and U. Kumar, 2017. Strategies for improving nitrogen use efficiency: A review. Agricultural Reviews, 38(1): 29-40.

Yao, X., J. Nie, R. Bai and X. Sui, 2020. Amino acid transporters in plants: Identification and function. Plants, 9(8): 972.

Zaman, I., M. Ali, K. Shahzad, M. S. Tahir, A. Matloob, W. Ahmad, S. Alamri, M. R. Khurshid, M. M. Qureshi and A. Wasaya, 2021. Effect of plant spacings on growth, physiology, yield and fiber quality attributes of cotton genotypes under nitrogen fertilization. Agronomy, 11(12): 2589.

Zayed, O., O. A. Hewedy, A. Abdelmoteleb, M. Ali, M. S. Youssef, A. F. Roumia, D. Seymour and Z.-C. Yuan, 2023. Nitrogen journey in plants: From uptake to metabolism, stress response, and microbe interaction. Biomolecules, 13(10): 1443.

Zhang, X., J. Wen, S. Jia, Y. He, W. Yang, W. Chen, D. Li, R. Liu, Q. Liu and Y. Cai, 2025. Glutamine synthetase ghgln1. 5 regulates arbuscular mycorrhizal symbiosis and verticillium wilt resistance in cotton by modulating inorganic nitrogen assimilation. New Phytologist, 246(2): 702-717.

Zhu, L., L. Liu, H. Sun, Y. Zhang, X. Liu, N. Wang, J. Chen, K. Zhang, Z. Bai and G. Wang, 2022. The responses of lateral roots and root hairs to nitrogen stress in cotton based on daily root measurements. Journal of agronomy and crop science, 208(1): 89-105.