

Case Study

Open Access

Breeding Rice Varieties for Low Nitrogen Environments

Qifu Zhang¹✉, Danyan Ding²¹ Hier Rice Research Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572025, Hainan, China;² Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, China✉ Corresponding email: qifu.zhang@hitar.orgMolecular Soil Biology, 2025, Vol.16, No.2 doi: [10.5376/msb.2025.16.0010](https://doi.org/10.5376/msb.2025.16.0010)

Received: 23 Feb, 2025

Accepted: 05 Apr., 2025

Published: 23 Apr., 2025

Copyright © 2025 Zhang and Ding, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:Zhang Q.F., and Ding D.Y., 2025, Breeding rice varieties for low nitrogen environments, Molecular Soil Biology, 16(2): 93-102 (doi: [10.5376/msb.2025.16.0010](https://doi.org/10.5376/msb.2025.16.0010))

Abstract Nitrogen is an essential nutrient for rice growth, but the excessive use of nitrogen fertilizers has led to environmental degradation, increased costs, and diminishing returns. Root structure and the ability to maintain chlorophyll content are key indicators of nitrogen use efficiency (NUE), while genes such as *OsTCP19*, *OsNAC68*, and *TOND1* play important roles in nitrogen uptake and assimilation. Field and hydroponic trials, combined with high-throughput phenotyping technologies, are effective in evaluating genotypic variation and selecting superior varieties. Breeding strategies include conventional selection, marker-assisted selection (MAS), genomic selection (GS), and the introgression of favorable traits from wild or traditional rice varieties. The case of Swarna-Sub1 demonstrates the potential of integrated breeding for enhancing both stress resistance and NUE. Despite significant progress, breeding efforts still face challenges such as balancing high yield with NUE, shortening breeding cycles, and adapting to diverse ecological zones. Integrating genetic improvement with sustainable agronomic practices—such as precision fertilization and organic fertilizer application—can reduce nitrogen input while maintaining yield, offering a promising path toward green, economical, and efficient rice production.

Keywords Nitrogen use efficiency (NUE); Low nitrogen stress; Rice breeding; Genome editing; Sustainable agriculture

1 Introduction

Nitrogen is one of the essential nutrients for rice and has a great impact on yield and quality. Since the "Green Revolution", the large-scale use of nitrogen fertilizers has indeed increased yields, but it has also caused problems such as environmental pollution, resource waste and rising costs (Lee, 2021; Wang et al., 2021). Therefore, improving the efficiency of nitrogen fertilizer use has become a key issue that needs to be urgently addressed in global rice cultivation.

The process of rice absorbing and utilizing nitrogen is controlled by multiple genes, involving root structure, related enzyme activity, and signal regulation mechanisms in the body (Li et al., 2022a; Lee, 2021; Kasemsap and Bloom, 2022). In recent years, with the development of molecular breeding and genomic technologies, researchers have found some genes and quantitative trait loci related to nitrogen efficiency (Li et al., 2022a; Xin et al., 2021; Liu et al., 2021).

In many developing countries, farmers often use nitrogen fertilizers poorly or even suffer from nitrogen shortages due to high prices, unstable supply or lack of technical guidance (Liu et al., 2021; Jyoti et al., 2024). Excessive use of nitrogen fertilizers not only increases economic pressure, but also easily causes environmental problems such as water pollution and greenhouse gas emissions (Lee, 2021; Wang et al., 2021). In areas with poor land and limited inputs, rice often faces low nitrogen stress (Castro-Pacheco et al., 2024; Jyoti et al., 2024). Some rice varieties can maintain good growth and yield even in the absence of sufficient nitrogen fertilizers. These varieties have strong low nitrogen tolerance (Liu et al., 2021; Ajmera et al., 2022; Qi et al., 2023; Zhou et al., 2025). This is mainly due to their more reasonable root structure, more efficient nitrogen absorption and assimilation ability. For example, optimizing the root angle, root number and distribution can significantly improve the absorption efficiency and yield performance in a low nitrogen environment (Ajmera et al., 2022; Zhou et al., 2025). At present, scientists have discovered some key genes and QTLs related to low nitrogen, such as *OsTCP19* and *OsNAC68*, which provide strong support for the breeding of new rice varieties with high nitrogen efficiency (Liu

et al., 2021; Xin et al., 2021; Li et al., 2022a). Promoting such low-nitrogen tolerant varieties can not only reduce the ecological pressure caused by nitrogen fertilizers, but also help farmers save costs, increase income, and promote the development of agriculture in a green and economical direction (Lee, 2021; Wang et al., 2021; Jyoti et al., 2024).

This study will focus on the latest progress in low-nitrogen rice breeding, focusing on the physiological and genetic response mechanisms of rice under low-nitrogen conditions, analyzing the genes and root phenotypic characteristics related to low-nitrogen traits, and introducing methods for germplasm screening and evaluation. At the same time, we will also pay attention to the performance of these results in actual production, and combine field performance, molecular marker selection, and genome analysis technologies to provide practical ideas and theoretical basis for low-nitrogen adaptive rice breeding.

2 Scientific BASIS for Rice's Low Nitrogen Tolerance

2.1 Physiological traits related to nitrogen use efficiency (NUE)

The length and thickness of rice roots directly affect its ability to absorb nitrogen from the soil. Studies have shown that in low nitrogen environments, the roots of certain rice genotypes can grow better and show stronger nitrogen absorption capacity (Li et al., 2022a). In particular, the larger the surface area of the root system and the more root hairs, the more conducive it is to absorb nutrients when nitrogen fertilizer is insufficient (Li et al., 2022a; Zhou et al., 2025).

Chlorophyll content reflects the photosynthesis level and nitrogen status of rice. When nitrogen is insufficient, leaves tend to turn yellow, chlorophyll decreases, and photosynthetic efficiency decreases. However, some low-nitrogen tolerant rice varieties can maintain a high green color and photosynthetic capacity even when nitrogen supply is low (Zhang et al., 2024; Qi et al., 2025; Xu et al., 2025). For example, rice expressing the maize *PCK2* gene can accumulate more photosynthetic pigments and biomass when nitrogen is insufficient, improving photosynthesis efficiency and nitrogen assimilation rate (Xu et al., 2025). In addition, these varieties also have advantages in terms of photosystem II efficiency and electron transfer rate (Zhang et al., 2024; Qi et al., 2025). In terms of yield, in a low-nitrogen environment, tolerant varieties can still maintain more tillers, longer panicles, and higher grain numbers, which are positively correlated with yield (Huang et al., 2022; Tao et al., 2022; Zhou et al., 2025). Some varieties can achieve good yield and harvest index even without nitrogen fertilizer (Huang et al., 2022; Tao et al., 2022). Whether rice can reasonably distribute nutrients, such as the ratio of roots to aboveground parts and the ratio of grains to straw, also determines its yield performance under nitrogen deficiency (Tao et al., 2022; Zhou et al., 2025).

2.2 Genetic regulation and QTL of nitrogen use efficiency

In rice, there are some proteins that specifically transport nitrogen, which are encoded by genes such as *NRT1.1B* and *OsNRT2*. These genes determine the ability of rice to absorb nitrate nitrogen and are important factors in improving nitrogen use efficiency (Wang et al., 2023; Li et al., 2022a; Zhang et al., 2015). *TOND1* is a key QTL. Studies have found that its overexpression can significantly improve rice yield and adaptability under nitrogen deficiency conditions (Zhang et al., 2015). Some genes related to purine metabolism, glycolysis and pentose phosphate pathways have also been found to be related to rice's low nitrogen adaptability (Wang et al., 2023).

Through QTL positioning, researchers have found many important regions related to low nitrogen tolerance, which control traits such as root length, biomass, and yield (Lian et al., 2005; Li et al., 2022a; 2022b). Some of these QTLs only work at specific nitrogen levels, indicating that rice's adaptation to low nitrogen is the result of the combined action of multiple genes (Lian et al., 2005). Molecular marker-assisted breeding technology has been used to screen low nitrogen-tolerant varieties and accelerate the gathering of excellent genes (Li et al., 2022a; 2022b).

The gene networks that regulate these traits are also very complex. For example, transcription factors such as NAC and TCP affect root development and nitrogen absorption (Nischal et al., 2012; Wang et al., 2023). The expression differences of some miRNAs also affect the response of rice to low nitrogen. The differences in

miRNA levels between different genotypes will change their adaptability to nitrogen stress (Nischal et al., 2012).

2.3 Screening and phenotypic identification strategies under low nitrogen environment

Field trials are currently the most commonly used method for low nitrogen tolerance screening. The general practice is to set up two treatments, low nitrogen and normal nitrogen, under the same conditions, and then compare the yield, tillering, panicle length and other indicators of different varieties to select materials with strong adaptability (Hu et al., 2015; Lestari et al., 2019a; Zhou et al., 2025). Combining cluster analysis and membership function method, the performance of each variety can be evaluated more systematically to find the most advantageous variety (Hu et al., 2015; Zhou et al., 2025). It is generally believed that theoretical yield, effective tiller number, ear length, and number of grains per ear are important indicators for measuring low nitrogen tolerance.

In addition to field trials, hydroponic and greenhouse trials are also often used for early screening and genetic research. In a controlled environment, root shape, biomass growth rate, and physiological changes can be clearly seen, which is very helpful for identifying genetic factors related to tolerance (Lian et al., 2005; Li et al., 2022b). The hydroponic system is particularly suitable for high-throughput screening and comparison between different genotypes, so it is also often used for QTL analysis and gene function verification.

With technological advances, phenomics has begun to be widely used in the screening process. By using automatic photography systems, drone remote sensing, multispectral imaging and other methods, multi-dimensional information such as leaf color, root length, and plant height of germplasm materials can be quickly collected, thereby improving screening efficiency and accuracy (Zhang et al., 2024; Zhou et al., 2025).

3 Breeding Methods for Low Nitrogen Tolerance

3.1 Conventional breeding strategies

Conventional breeding is the basic method for breeding low nitrogen tolerant rice. The practice is usually to plant a large number of germplasm materials in low nitrogen fields for observation, and then select varieties with better performance as parental pairs for hybridization. Then find plants that grow well under low nitrogen conditions from the offspring, select them repeatedly for several generations, and keep low nitrogen tolerant individuals. Studies have found that using traits such as biological yield, tiller number, panicle length and panicle number as screening indicators can effectively find germplasm that performs well under low nitrogen (Lestari et al., 2019b; Zhou et al., 2025). Continuing to select in a low nitrogen environment in each generation helps to accumulate favorable genes and improve the performance of offspring (Lestari et al., 2019b). In addition, statistical tools such as cluster analysis and membership function method can also be used to help comprehensively judge which materials are more tolerant to low nitrogen and improve efficiency (Zhou et al., 2025).

Farmer-participated variety selection (FPVS) emphasizes combining actual production needs. At the breeding site, farmers directly participate in the screening process and evaluate which varieties are more stable and adaptable under low nitrogen based on their own experience. This method can ensure that the selected materials are closer to production practice and convenient for subsequent promotion and use (Vinod and Heuer, 2012).

3.2 Marker-assisted selection (MAS) and genomic selection (GS)

Marker-assisted selection (MAS) is an important means of selecting rice at the genetic level. Researchers have found many QTL loci and genes related to low nitrogen tolerance and nitrogen use efficiency (NUE) (Lian et al., 2005; Wei et al., 2012; Shen et al., 2021; Li et al., 2022a; 2022b). With these markers, plants carrying good genes can be quickly selected in early breeding, saving time. For example, loci and genes such as *TOND1*, *OsLHT1*, and *OsACR9* have been confirmed to be associated with low nitrogen and can be used as key targets in actual breeding (Wang et al., 2023; Shen et al., 2021). In addition, fine positioning using near-isogenic lines or recombinant inbred line populations can help to more accurately identify the genes that control these traits (Lian et al., 2005; Wei et al., 2012; Shen et al., 2021).

Genomic selection (GS) is a further approach. Instead of looking at just a few genes, it analyzes the entire genome

map and inputs the genotype and trait data into the model to predict the breeding value of each plant. This can consider the contributions of major effect genes and small effect genes at the same time, which is more suitable for complex traits, such as low nitrogen tolerance (Vinod and Heuer, 2012; Li et al., 2022b). As long as the model is well established, it is not necessary to conduct multi-generation field trials every time. It is possible to directly estimate which materials have greater potential in the early stage, significantly shortening the breeding cycle (Li et al., 2022b).

3.3 Introduction of traditional or wild rice traits

Wild rice, such as *Oryza rufipogon*, is rich in genetic diversity and is a treasure trove of stress resistance traits. Introducing its good genes into cultivated rice through hybridization can effectively improve the adaptability to low nitrogen and the efficiency of nitrogen utilization (Wang et al., 2023). Through multi-omics analysis, it was found that a group of genes in wild rice are related to nitrogen metabolism, energy pathways and signal transduction, and these genes can be used as new breeding materials (Shen et al., 2021; Wang et al., 2023).

Commonly used methods are hybridization or backcrossing. First, hybridize wild rice or traditional low-nitrogen tolerant varieties with cultivated rice to select plants with target traits. Then, through backcrossing, the background can be slowly returned to cultivated rice while retaining the gene for low nitrogen tolerance (Feng et al., 2018; Shen et al., 2021; Wang et al., 2023). It is also possible to use near-isogenic lines and specific introgression lines to combine multiple stress tolerance traits, such as drought tolerance and low nitrogen tolerance, into one variety (Feng et al., 2018).

4 Case Study: Development and Adoption of “Swarna-Sub1” and its Derivatives

4.1 Breeding background and process

Swarna is a high-yield variety widely planted in eastern India, but its yield is reduced or even lost when it encounters floods. FR13A is a traditional rice variety that can survive flooding for more than ten days and is a representative of flood resistance research. The goal of breeders is to introduce the flood tolerance gene (Sub1A) of FR13A into Swarna, retaining the high-yield characteristics of Swarna while enhancing its flood resistance. They used molecular marker-assisted backcrossing (MAB) technology to ensure that only useful genes are introduced and other traits remain unchanged as much as possible (Neeraja et al., 2007; Mohapatra et al., 2021).

Researchers also added genes for resistance to bacterial leaf streak and blast to Swarna-Sub1, improving its disease resistance and stability in different environments (Patroti et al., 2019; Mohapatra et al., 2021).

In addition to flood resistance, breeders also pay special attention to its performance under low nitrogen conditions. They selected offspring under different nitrogen fertilizer levels and picked out plants that used less nitrogen fertilizer and had higher yields. The results showed that Swarna-Sub1 still grew well in a nitrogen-deficient environment, breaking the limitation of "low nitrogen means reduced yield" (Bhowmick et al., 2014; Singh et al., 2023).

4.2 Performance in low nitrogen environment

Field trial data showed that Swarna-Sub1 had an average yield of 4.5~4.8 tons/hectare in flood years, which was significantly higher than ordinary varieties (Mishra and Kar, 2020; Singh et al., 2023) (Figure). With less fertilizer, it can still maintain a high number of tillers and biomass, and the output per unit of nitrogen fertilizer is higher (Bhowmick et al., 2014; Singh et al., 2023).

In contrast, although traditional flood-tolerant varieties can survive, their yields are low; ordinary high-yield varieties directly reduce their yields when they encounter flooding. Swarna-Sub1 does better in both aspects. In flood years, it can yield more than double that of old varieties, and in normal years, its yield is on par with mainstream varieties (Dar et al., 2013; 2017; Singh et al., 2023). Under nitrogen-deficient conditions, its nitrogen utilization efficiency is also higher, and more food is produced with less fertilizer (Dar et al., 2013; Mishra and Kar, 2020; Singh et al., 2023).



Figure 1 (A) Comparative lesion length of donor parent and pyramided lines; (B) bacterial blight disease inoculated pyramided plant; and (C,D) panicles of donor parents and pyramided lines (Adopted from Mishra and Kar, 2020)

4.3 Application and effect by farmers

Swarna-Sub1 has been rapidly promoted in flood-prone areas such as India and Bangladesh. For example, in 2013, Odisha distributed seeds to 355 farmers, and one year later, 75% of farmers took the initiative to continue planting, indicating that everyone recognized this variety (Dar et al., 2017). Statistics show that in flood years, farmers who planted Swarna-Sub1 saw an average increase in yield of 19% and an increase in income of 48.2%, which is significantly higher than farmers who did not use this variety (Dar et al., 2013; 2017; Raghu et al., 2022).

For some farmers who have poor farming conditions and are often affected by floods (such as low-caste farmers), the benefits brought by this variety are even more obvious. Their land is low-lying and easily flooded, and Swarna-Sub1 has helped them stabilize their harvests, increase food security, and increase their income (Dar et al., 2013; 2017).

Its high nitrogen efficiency means that less fertilizer can be applied without affecting yields, which can save farmers money on fertilizers and reduce environmental pollution (Bhowmick et al., 2014; Singh et al., 2023). Field trials and farmer feedback have shown that Swarna-Sub1 can still produce high yields when nitrogen is deficient and can maintain stable yields with less fertilizer, which helps reduce eutrophication of water bodies and greenhouse gas emissions. At the same time, this also promotes the development of green agriculture and makes agriculture more sustainable (Bhowmick et al., 2014; Dar et al., 2017; Singh et al., 2023).

5 Comparative Analysis: IRRI-Led Low-Nitrogen Trials in Southeast Asia

5.1 Basic information on IRRI multi-site trials

The International Rice Research Institute (IRRI) has set up low-nitrogen trial sites in many countries in Southeast Asia, covering the main rice-growing areas of Myanmar, Indonesia, Thailand and Vietnam. The climate, soil type and planting management level of these locations vary. For example, Bago in Myanmar, Yogyakarta in Indonesia, Nakhon Sawan in Thailand and Can Tho in Vietnam all have trial fields to observe the performance of different varieties and fertilization methods under low-nitrogen conditions (Witt et al., 1999; Silva et al., 2022).

All trial sites use a unified method to focus on recording data on nitrogen fertilizer application, yield, nitrogen use efficiency, crop traits and farmer feedback. The experimental design includes two schemes: conventional fertilization and low-nitrogen treatment. The differences between varieties are compared through on-site yield

measurement, soil and plant analysis. At the same time, local farmers also participated in observing and evaluating the actual performance of the varieties (Witt et al., 1999; Silva et al., 2022). In some areas, the experiment also cooperated with field schools and technical observation activities to improve farmers' understanding and operational capabilities of low-nitrogen management (Silva et al., 2022; Suman et al., 2024).

5.2 Results of farmers' participation in variety screening

In these experiments, farmers directly participated in variety selection activities and achieved good results. For example, in Indonesia and Vietnam, farmers selected new varieties with stable yields and strong adaptability under low nitrogen by visiting the site and evaluating varieties (Silva et al., 2022). In Bago, Myanmar, the yield difference between the low-nitrogen treatment group and the control group was large, indicating that the region still has potential for improvement; while in Can Tho, Vietnam, the yield difference was small, indicating that there are good management measures and limited room for improvement.

Farmers not only value yield, but also compare other characteristics such as disease resistance, rice quality, length of harvest period and whether it is easy to manage (Silva et al., 2022). From the feedback, saving nitrogen fertilizer, reducing costs and improving benefits are important reasons for them to choose new varieties (Silva et al., 2022; Suman et al., 2024). Their enthusiasm for participation also makes these new technologies landed faster and promoted more smoothly in the local area (Suman et al., 2024).

5.3 Promotion policies and supporting measures

To promote the application of low-nitrogen varieties, governments encourage farmers to use these technologies through subsidies, training and promotion activities (Begho et al., 2022). Studies have shown that education and agricultural technical services, coupled with necessary financial support, are important factors affecting whether farmers adopt new technologies. IRRI also cooperates with research institutions in various countries to promote farmer field schools, set up demonstration sites, and compile technical materials to help farmers master low-nitrogen management methods (Begho et al., 2022; Suman et al., 2024).

There are still problems with seed promotion. The main difficulties are insufficient seeds of excellent varieties, imperfect distribution channels, and some farmers are unfamiliar with new varieties (Begho et al., 2022). To solve these problems, it is necessary to strengthen the connection between seed companies and farmers, improve the seed certification system and circulation system, and ensure that farmers can easily buy suitable seeds.

6 Challenges and Limitations of Breeding in Low Nitrogen Environments

6.1 The contradiction between yield potential and nitrogen use efficiency (NUE)

In crops such as rice, improving nitrogen use efficiency (NUE) requires improving nitrogen absorption, transport and utilization, which are regulated by complex genetic and physiological mechanisms (Van Bueren and Struik, 2017; Ranjan and Yadav, 2020; Chen et al., 2022). Although there is a certain amount of genetic variation in modern crop varieties that can be used to improve NUE, most of the related traits are quantitative traits controlled by multiple genes and are difficult to improve quickly through a single method (Cormier et al., 2016).

In low nitrogen environments, the heritability of crop yield is lower than that under high nitrogen conditions. This phenomenon is partly due to the smaller differences between genotypes, which affects the efficiency of selection (Bänziger et al., 1997; Ertiro et al., 2020). For example, in maize, yield heritability under low nitrogen conditions is 29% lower than that under high nitrogen conditions (Bänziger et al., 1997). Traits closely related to NUE, such as root activity and nitrogen redistribution, are more critical in low nitrogen environments, but these traits are difficult to phenotype and genetic improvement is slower (Ranjan and Yadav, 2020; Chen et al., 2022).

In practical applications, farmers want varieties to maintain high yields under low nitrogen conditions and also require them to maintain high yields in high nitrogen environments. However, some studies have shown that as the degree of nitrogen stress increases, the correlation between low nitrogen and high nitrogen yields weakens, indicating that some genotypes perform inconsistently under different nitrogen conditions (Begho et al., 2022; Amegbor et al., 2022x). There are few varieties that can perform well under both conditions, and breeding must

make a trade-off between high yield and high NUE (Van Bueren and Struik, 2017; Neuweiler et al., 2021).

Gene or QTL expression often varies between nitrogen environments, with some being condition-specific, which complicates breeding across multiple environments (Garoma et al., 2021; Chen et al., 2022).

6.2 Long breeding cycles and high resource requirements

Breeding under low-nitrogen conditions demands multi-location, multi-year trials across varying nitrogen levels to accurately compare genotypes for yield and NUE performance (Zhao et al., 2019; Ertiro et al., 2020). These efforts require significant investment and organizational capacity, particularly in terms of funding and infrastructure (Chen et al., 2022).

Molecular breeding methods such as genomic selection and marker-assisted selection also depend on high-throughput genotyping and phenotyping platforms, which are often burdensome for institutions in developing countries (Badu-Apraku and Fakorede, 2017). Establishing and maintaining low-nitrogen screening fields adds to ongoing costs (Van Bueren and Struik, 2017).

Most NUE-related traits are quantitative and strongly influenced by the environment. Introducing these traits into elite cultivars often requires multiple generations of selection and backcrossing (Kamara et al., 2024). Traits like root structure or nitrogen remobilization are difficult to observe in early generations, and selection often occurs only in later stages, further prolonging breeding cycles (Ertiro et al., 2020; Ranjan and Yadav, 2020).

6.3 Difficulty in adapting to diverse ecological zones

Genotype-by-environment interactions (G×E) are a persistent challenge in breeding and are especially significant in low-nitrogen breeding (Van Bueren and Struik, 2017). A variety may perform well under specific soil, climate, and management conditions but not in others (Ertiro et al., 2020; Kimutai et al., 2021).

Systematic evaluation across multiple locations and years is necessary to identify genotypes with either broad stability or strong adaptation to specific environments (Bänziger et al., 1997). This requires extensive environmental testing at every breeding stage, which increases both time and cost.

Certain NUE-related QTLs or genes may only be effective under specific nitrogen levels, and their functionality may be lost in other settings (Garoma et al., 2021). This limits gene selection and utilization and adds uncertainty to variety development (Neuweiler et al., 2021; Chen et al., 2022).

Some genotypes that perform well under low nitrogen may underperform under high nitrogen or in response to other stresses such as drought or disease, making them unsuitable for broad deployment (Makinde et al., 2023). Therefore, breeding efforts must consider not only NUE but also stress resistance and yield stability (Ertiro et al., 2020; Kimutai et al., 2021).

7 Future Development Directions and New Opportunities

7.1 Application of CRISPR and genome editing in rice NUE gene improvement

CRISPR/Cas9 and other tools have been widely used in rice breeding in recent years, especially in improving nitrogen use efficiency (NUE). Researchers have found many genes related to nitrogen absorption and utilization, such as nitrogen transporters, nitrogen metabolism-related enzymes, and regulatory factors (Fiaz et al., 2021; Hu et al., 2022; Wang et al., 2022). Knocking out, inserting or precisely editing these genes with CRISPR can change gene expression and thus improve nitrogen absorption capacity. For example, editing genes such as OsNRT1.1B and OsNLP4 has significantly enhanced the nitrogen uptake and utilization efficiency of rice (Yu et al., 2020; Fiaz et al., 2021).

This technology can not only modify one gene, but also manipulate multiple genes at the same time, breaking through the previous limitation of only being able to improve a single trait and speeding up the breeding process (Fiaz et al., 2021; Hu et al., 2022). There are also newer tools, such as base editing and in situ editing, which can regulate DNA more finely and have higher operating efficiency, and are suitable for dealing with complex traits involving multiple genes such as NUE (Fiaz et al., 2021). However, this technology has not yet been promoted on

a large scale. Some countries treat gene-edited crops the same as genetically modified crops, with strict approval, resulting in a slow pace of new varieties being brought to market. In order to implement these results, the policy level needs to make a distinction, clarify which ones belong to traditional genetic modification and which ones are editing breeding, and reduce social concerns. In addition, detection methods, field testing methods and seed management systems must be established to ensure the stability and safety of these varieties (Fiaz et al., 2021; Hu et al., 2022).

7.2 Combination with sustainable agriculture

Improving NUE cannot rely solely on genetic modification, but also requires reasonable planting management measures, such as precision fertilization, use of slow-release fertilizers, and adjustment of fertilization locations (Hu et al., 2022; Wang et al., 2022). In practice, combining efficient nitrogen utilization varieties with these technologies can further reduce the use of chemical fertilizers while maintaining yields (Wang et al., 2022; Jyoti et al., 2024). Breeding varieties with strong root systems and combining them with organic fertilizers can increase the release rate of nitrogen in the soil, allowing crops to grow normally in low-nitrogen environments (Ajmera et al., 2022; Wang et al., 2022).

In the future, it is necessary to strengthen the coordination of breeding and agronomic technology, develop local cultivation models for different climate and soil conditions, and achieve the goal of high yields with less fertilizer (Wang et al., 2022; Jyoti et al., 2024).

This type of low-nitrogen and high-efficiency variety is very suitable for organic agriculture or ecological planting methods. Combining traditional methods such as green manure, intercropping, and organic fertilizers with varieties with strong nitrogen absorption capacity can not only improve the overall nitrogen utilization efficiency of the system, but also reduce pollution and greenhouse gas emissions (Ajmera et al., 2022; Jyoti et al., 2024). Next, we should focus on promoting this low-input, high-efficiency green planting system to promote sustainable agricultural development (Wang et al., 2022; Jyoti et al., 2024).

7.3 Application of digital technology and AI in rice breeding

Current remote sensing, drones, and digital phenotyping platforms are developing rapidly, which can efficiently record the growth of rice, such as plant growth, leaf color, root status, etc. (Ajmera et al., 2022; Salama et al., 2024). These devices can quickly collect a large amount of information and can be used repeatedly in different locations and at different times, without being restricted by weather and labor, which improves the accuracy and efficiency of phenotypic analysis. In the future, these technologies will become important tools for low-nitrogen rice breeding.

Big data and artificial intelligence also play an important role in analyzing these data. Combining genetic data, phenotypic data, soil environment, field management and other information, AI algorithms can be used to screen genotypes with good performance and predict the yield performance of new varieties under various nitrogen levels. Machine learning models can also help breeders optimize breeding routes, such as how to combine genes, in which areas to test, and how to arrange field trials.

Acknowledgments

We sincerely thank the two anonymous reviewers for their valuable opinions and suggestions.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Ajmera I., Henry A., Radanielson A., Klein S., Ianevski A., Bennett M., Band L., and Lynch J., 2022, Integrated root phenotypes for improved rice performance under low nitrogen availability, *Plant, Cell & Environment*, 45: 805-822.
<https://doi.org/10.1111/pce.14284>

- Amegbor I., Abe A., Adjebeng-Danquah J., and Adu G., 2022, Genetic analysis and yield assessment of maize hybrids under low and optimal nitrogen environments, *Heliyon*, 8(3): e09052.
<https://doi.org/10.1016/j.heliyon.2022.e09052>
- Badu-Apraku B., and Fakorede M., 2017, Breeding for tolerance to low soil nitrogen, in *advances in genetic enhancement of early and extra-early maize for Sub-Saharan Africa*, Cham: Springer International Publishing, pp.359-378.
https://doi.org/10.1007/978-3-319-64852-1_13
- Bänziger M., Betrán F., and Lafitte H., 1997, Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments, *Crop Science*, 37: 1103-1109.
<https://doi.org/10.2135/CROPSCI1997.0011183X003700040012X>
- Begho T., Glenk K., Anik A., and Eory V., 2022, A systematic review of factors that influence farmers' adoption of sustainable crop farming practices: Lessons for sustainable nitrogen management in South Asia, *Journal of Sustainable Agriculture and Environment*, 1(2): 149-160.
<https://doi.org/10.1002/sae2.12016>
- Bhowmick M., Dhara M., Singh S., Dar M., and Singh U., 2014, Improved management options for submergence-tolerant (Sub1) rice genotype in flood-prone rainfed lowlands of west bengal, *American Journal of Plant Sciences*, 5: 14-23.
<https://doi.org/10.4236/AJPS.2014.51003>
- Castro-Pacheco S., Rabekijana R., Andriamiarana M., Raveloson H., Rakotomalala J., Ramanantsoanirina A., Garin V., Grenier C., and Brocke K., 2024., Participatory Plant Breeding to develop biofortified upland rice for marginal environments, *Experimental Agriculture*, 60: e26.
<https://doi.org/10.1017/S0014479724000218>
- Chen Z., Li L., Halford N., Xu H., Huang L., Gao R., Lu R., and Liu C., 2022, Advances in barley breeding for improving nitrogen use efficiency, *Agronomy*, 12(7): 1682.
<https://doi.org/10.3390/agronomy12071682>
- Cormier F., Foulkes J., Hirel B., Gouache D., Moënné-Loccoz Y., and Gouis L., 2016, Breeding for increased nitrogen-use efficiency: a review for wheat (*T. aestivum* L.), *Plant Breeding*, 135: 255-278.
<https://doi.org/10.1111/PBR.12371>
- Dar M., Chakravorty R., Waza S., Sharma M., Zaidi N., Singh A., Singh U., and Ismail A., 2017, Transforming rice cultivation in flood prone coastal Odisha to ensure food and economic security, *Food Security*, 9: 711-722.
<https://doi.org/10.1007/s12571-017-0696-9>
- Dar M., De Janvry A., Emerick K., Raitzer D., and Sadoulet E., 2013, Flood-tolerant rice reduces yield variability and raises expected yield, differentially benefitting socially disadvantaged groups, *Scientific Reports*, 3(1): 3315.
<https://doi.org/10.1038/srep03315>
- Ertiro B., Labuschagne M., Olsen M., Das B., Prasanna B., and Gowda M., 2020, Genetic dissection of nitrogen use efficiency in tropical maize through genome-wide association and genomic prediction, *Frontiers in Plant Science*, 11: 474.
<https://doi.org/10.3389/fpls.2020.00474>
- Feng B., Chen K., Cui Y., Wu Z., Zheng T., Zhu Y., Ali J., Wang B., Xu J., Zhang W., and Li Z., 2018, Genetic dissection and simultaneous improvement of drought and low nitrogen tolerances by designed QTL pyramiding in rice, *Frontiers in Plant Science*, 9: 306.
<https://doi.org/10.3389/fpls.2018.00306>
- Fiaz S., Wang X., Khan S., Ahmar S., Noor M., Riaz A., Ali K., Abbas F., Mora-Poblete F., Figueroa C., and Alharthi B., 2021, Novel plant breeding techniques to advance nitrogen use efficiency in rice: A review, *GM Crops & Food*, 12: 627-646.
<https://doi.org/10.1080/21645698.2021.1921545>
- Garoma B., Alamirew S., and Chibsa T., 2021, Review on genetic and breeding for low -N tolerance in maize, *Journal of Natural Sciences Research*, 12: 26-31.
<https://doi.org/10.7176/jnsr/12-1-04>
- Hu B., Li X., Wan Y., Qiu Z., Nie Y., and Xie J., 2015, Index screening and comprehensive evaluation of phenotypic traits of low nitrogen tolerance using BILs population derived from Dongxiang wild rice (*Oryza rufipogon* Griff), *Ying yong sheng tai xue bao = The journal of applied ecology*, 26(8): 2346-2352.
- Hu B., Wang W., Chen J., Liu Y., and Chu C., 2022, Genetic improvement toward nitrogen-use efficiency in rice: lessons and perspectives, *Molecular plant*, 16(1): 64-74.
<https://doi.org/10.1016/j.molp.2022.11.007>
- Huang M., Liu Y., Chen J., and Cao F., 2022, The difference method is not necessarily reliable for comparing the nitrogen use efficiency of hybrid rice cultivars with different tolerance to low nitrogen conditions, *Crop and Environment*, 1(3): 168-172.
<https://doi.org/10.1016/j.crope.2022.08.003>
- Jyoti S., Singh G., Pradhan A., Tarpley L., Septiningsih E., and Talukder S., 2024, Rice breeding for low input agriculture, *Frontiers in Plant Science*, 15: 1408356.
<https://doi.org/10.3389/fpls.2024.1408356>
- Kamara M., Mansour E., Khalaf A., Eid M., Hassanin A., Abdelghany A., Kheir A., Galal A., Behiry S., Silvar C., and El-Hendawy S., 2024, Molecular diversity and combining ability in newly developed maize inbred lines under low-nitrogen conditions, *Life*, 14(5): 641.
<https://doi.org/10.3390/life14050641>
- Kasemsap P., and Bloom A., 2022, Breeding for higher yields of wheat and rice through modifying nitrogen metabolism, *Plants*, 12(1): 85.
<https://doi.org/10.3390/plants12010085>

- Kimutai C., Gowda M., and Kiplagat O., 2021, Trait-QTL-heritability of grain yield and other agronomic traits under low nitrogen conditions in bi-parental maize populations, *International Journal of Biological and Pharmaceutical Sciences Archive*, 2(1): 96-116.
<https://doi.org/10.53771/ijbpsa.2021.2.1.0072>
- Lee S., 2021. Recent advances on nitrogen use efficiency in rice, *Agronomy*, 11: 753.
<https://doi.org/10.3390/AGRONOMY11040753>
- Lestari A. P., Sopandie D., and Aswidinnoor H., 2019a, Estimation for stress tolerance indices of rice genotypes in low nitrogen condition, *Thai Journal of Agricultural Science*, 52(4): 180-190.
- Lestari A., Suwarno S., Trikoesoemaningtyas T., Sopandie D., and Aswidinnoor H., 2019b, The tolerance and yield components of rice breeding lines selected under low and optimum nitrogen conditions, *Indonesian Journal of Agricultural Science*, 20(2): 61-68.
<https://doi.org/10.21082/ijas.v20n2.2019.p61-68>
- Li J., Xin W., Wang W., Zhao S., Xu L., Jiang X., Duan Y., Zheng H., Yang L., Liu H., Jia Y., Zou D., and Wang J., 2022a, Mapping of candidate genes in response to low nitrogen in rice seedlings, *Rice*, 15(1): 51.
<https://doi.org/10.1186/s12284-022-00597-x>
- Li Q., Lu X., Wang C., Shen L., Dai L., He J., Yang L., Li P., Hong Y., Zhang Q., Dong G., Hu J., Zhang G., Ren D., Gao Z., Guo L., Qian Q., Zhu L., and Zeng D., 2022b, Genome-wide association study and transcriptome analysis reveal new QTL and candidate genes for nitrogen-deficiency tolerance in rice., *The Crop Journal*, 10(4): 942-951.
<https://doi.org/10.1016/j.cj.2021.12.006>
- Lian X., Xing Y., Yan H., Xu C., Li X., and Zhang Q., 2005, QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid, *Theoretical and Applied Genetics*, 112: 85-96.
<https://doi.org/10.1007/s00122-005-0108-y>
- Liu Y., Wang H., Jiang Z., Wang W., Xu R., Wang Q., Zhang Z., Li A., Liang Y., Ou S., Liu X., Cao S., Tong H., Wang Y., Zhou F., Liao H., Hu B., and Chu C., 2021, Genomic basis of geographical adaptation to soil nitrogen in rice, *Nature*, 590: 600-605.
<https://doi.org/10.1038/s41586-020-03091-w>
- Makinde S., Badu-Apraku B., Ariyo O., and Porbeni J., 2023, Combining ability of extra-early maturing pro-vitamin A maize (*Zea mays* L.) inbred lines and performance of derived hybrids under *Striga hermonthica* infestation and low soil nitrogen, *PLOS ONE*, 18(2): e0280814.
<https://doi.org/10.1371/journal.pone.0280814>
- Mishra S., and Kar M., 2020, Biochemical performance of some Sub1 rice (*Oryza sativa*) genotypes under submergence stress in Odisha, *International Journal of Chemical Studies*, 8(4): 1709-1716.
<https://doi.org/10.22271/CHEMI.2021.V9.I2L.11922>
- Mohapatra S., Panda A., Bastia A., Mukherjee A., Sanghamitra P., Meher J., Mohanty S., and Pradhan S., 2021, Development of submergence-tolerant, bacterial blight-resistant, and high-yielding near isogenic lines of popular variety, 'Swarna' through marker-assisted breeding approach, *Frontiers in Plant Science*, 12: 672618.
<https://doi.org/10.3389/fpls.2021.672618>
- Neeraja C., Neeraja C., Maghirang-Rodriguez R., Pamplona A., Heuer S., Collard B., Septiningsih E., Vergara G., Sanchez D., Xu K., Ismail A., and Mackill D., 2007, A marker-assisted backcross approach for developing submergence-tolerant rice cultivars, *Theoretical and Applied Genetics*, 115: 767-776.
<https://doi.org/10.1007/s00122-007-0607-0>
- Neuweiler J., Trini J., Maurer H., and Würschum T., 2021, Do lower nitrogen fertilization levels require breeding of different types of cultivars in triticale? TAG. *Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, 135: 993-1009.
<https://doi.org/10.1007/s00122-021-04012-9>
- Nischal L., Mohsin M., Khan I., Kardam H., Wadhwa A., Abrol Y., Iqbal M., and Ahmad A., 2012, Identification and comparative analysis of micrnas associated with low-N tolerance in rice genotypes, *PLoS ONE*, 7(12): e50261.
<https://doi.org/10.1371/journal.pone.0050261>
- Patroti P., Vishalakshi B., Umakanth B., Suresh J., Senguttuvel P., and Madhav M., 2019. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1, an elite rice variety (*Oryza sativa* L.). *Euphytica*, 215(11): 179.
<https://doi.org/10.1007/s10681-019-2487-1>
- Qi Z., Ling F., Jia D., Cui J., Zhang Z., Xu C., Yu L., Guan C., Wang Y., Zhang M., and Dou J., 2023, Effects of low nitrogen on seedling growth, photosynthetic characteristics and antioxidant system of rice varieties with different nitrogen efficiencies, *Scientific Reports*, 13(1): 19780.
<https://doi.org/10.1038/s41598-023-47260-z>
- Qi Z., Xu C., Tang R., Zhang Q., Sun W., Guan C., Wang Y., Zhang M., Ding J., Zhang Y., Yang H., Yang Y., Liu X., Zhang Z., and Ling F., 2025. Response of Photosynthesis and Chlorophyll Fluorescence to Nitrogen Changes in Rice with Different Nitrogen Use Efficiencies. *Plants*, 14(10): 1465.
<https://doi.org/10.3390/plants14101465>
- Raghu P., Veetil P., and Das S., 2022, Smallholder adaptation to flood risks: Adoption and impact of Swarna-Sub1 in eastern India, *Environmental Challenges*, 7: 100480.
<https://doi.org/10.1016/j.envc.2022.100480>
- Ranjan R., and Yadav R., 2020, Genetics analysis of nitrogen use efficiency component traits under nitrogen-limiting environment, *Cereal Research Communications*, 48: 431-439.
<https://doi.org/10.1007/s42976-020-00063-8>

- Rao I., Neeraja C., Srikanth B., Subrahmanyam D., Swamy K., Rajesh K., Vijayalakshmi P., Kiran T., Sailaja N., Revathi P., Rao P., Rao L., Surekha K., Babu V., and Voleti S., 2018, Identification of rice landraces with promising yield and the associated genomic regions under low nitrogen, Scientific Reports, 8(1): 9200.
<https://doi.org/10.1038/s41598-018-27484-0>
- Salama E., Kambale R., Mohan S., Premnath A., Yousef A., Moursy A., Abdelsalam N., Moneim D., Muthurajan R., and Boopathi N., 2024, Empowering rice breeding with NextGen genomics tools for rapid enhancement nitrogen use efficiency, Gene, 927: 148715.
<https://doi.org/10.1016/j.gene.2024.148715>
- Shen C., Chen K., Cui Y., Chen J., Mi X., Zhu S., Zhu Y., Ali J., Ye G., Li Z., and Xu J., 2021, QTL mapping and favorable allele mining of nitrogen deficiency tolerance using an interconnected breeding population in rice, Frontiers in Genetics, 12: 616428.
<https://doi.org/10.3389/fgene.2021.616428>
- Silva J., Pede V., Radanielson A., Kodama W., Duarte A., De Guia A., Malabayabas A., Pustika A., Argosubekti N., Vithoonjit D., Hieu P., Pame A., Singleton G., and Stuart A., 2022, Revisiting yield gaps and the scope for sustainable intensification for irrigated lowland rice in Southeast Asia, Agricultural Systems, 198: 103383.
<https://doi.org/10.1016/j.agsy.2022.103383>
- Singh R., Singh P., Singh H., Christopher K., Singh P., Kumar A., and Kumar A., 2023, Impact of climate resilient technology on growth and yield of paddy (*Oryza sativa* L.) under submergence condition, International Journal of Environment and Climate Change, 13(10): 3057-3065.
<https://doi.org/10.9734/ijec/2023/v13i102974>
- Suman A., Mishra A., Shukla G., Sah D., Chandra U., Chaubey A., Mishra B., Pathak J., and Panwar G., 2024, Analyzing alternatives for managing nitrogen in puddled transplanted rice in a semi-arid area of India, Sustainability, 16(14): 6096.
<https://doi.org/10.3390/su16146096>
- Tao Z., Liu Y., Chen J., Cao F., and Huang M., 2022, Yield attributes response to nitrogen fertilization in low-nitrogen tolerant hybrid rice, Agronomy, 12(10), 2320.
<https://doi.org/10.3390/agronomy12102320>
- Van Bueren E., and Struik P., 2017, Diverse concepts of breeding for nitrogen use efficiency. A review, Agronomy for Sustainable Development, 37: 1-24.
<https://doi.org/10.1007/s13593-017-0457-3>
- Vinod K., and Heuer S., 2012, Approaches towards nitrogen- and phosphorus-efficient rice. AoB Plants, 2012: pls028.
<https://doi.org/10.1093/aobpla/pls028>
- Wang B., Zhou G., Guo S., Li X., Yuan J., and Hu A., 2022, Improving nitrogen use efficiency in rice for sustainable agriculture: strategies and future perspectives, Life, 12(10): 1653.
<https://doi.org/10.3390/life12101653>
- Wang F., Yoshida H., and Matsuoka M., 2021, Making the "green revolution" truly green: improving crop nitrogen use efficiency, Plant & cell physiology, 62(6): 942-947.
<https://doi.org/10.1093/pcp/pcab051>
- Wang Y., Jiang J., Qian Y., Miao S., Wang W., Xu J., Fu B., Zhang F., and Zhao X., 2023, Multi-omics analysis reveals the regulatory and metabolic mechanisms underlying low-nitrogen tolerance at the flowering stage in rice, Agronomy, 13(2): 578.
<https://doi.org/10.3390/agronomy13020578>
- Wei D., Cui K., Ye G., Pan J., Xiang J., Huang J., and Nie L., 2012, QTL mapping for nitrogen-use efficiency and nitrogen-deficiency tolerance traits in rice, Plant and Soil, 359: 281-295.
<https://doi.org/10.1007/s11104-012-1142-6>
- Witt C., Dobermann A., Abdurachman S., Gines H., Wang G., Nagarajan R., Satawatananont S., Son T., Tan P., Van Tiem L., Simbahan G., and Olk D., 1999, Internal nutrient efficiencies of irrigated lowland rice in tropical and subtropical Asia, Field Crops Research, 63: 113-138.
[https://doi.org/10.1016/S0378-4290\(99\)00031-3](https://doi.org/10.1016/S0378-4290(99)00031-3)
- Xin W., Wang J., Li J., Zhao H., Liu H., Zheng H., Yang L., Wang C., Yang F., Chen J., and Zou D., 2021, Candidate gene analysis for nitrogen absorption and utilization in japonica rice at the seedling stage based on a genome-wide association study, Frontiers in Plant Science, 12: 670861.
<https://doi.org/10.3389/fpls.2021.670861>
- Xu H., Zhang H., Xu Y., Li C., Liu X., Wu T., Zong L., Jiang D., Chen G., and Gao Z., 2025, Overexpressed phosphoenolpyruvate carboxykinase 2 (PCK2) from maize in rice enhances tolerance to low nitrogen stress, Plant Growth Regulation, 105(1): 257-272.
<https://doi.org/10.1007/s10725-024-01224-z>
- Yu J., Xuan W., Tian Y., Fan L., Sun J., Tang W., Chen G., Wang B., Liu Y., Wu W., Liu X., Jiang X., Zhou C., Dai Z., Xu D., Wang C., and Wan J., 2020, Enhanced OsNLP4-OsNiR cascade confers nitrogen use efficiency by promoting tiller number in rice, Plant Biotechnology Journal, 19: 167-176.
<https://doi.org/10.1111/pbi.13450>
- Zhang Y., Cai H., You E., Qiao X., Gao Z., and Chen G., 2024, Physiological response to low-nitrogen stress and comprehensive evaluation in four rice varieties, Photosynthetica, 62: 252-262.
<https://doi.org/10.32615/ps.2024.028>
- Zhang Y., Tan L., Zhu Z., Yuan L., Xie D., and Sun C., 2015, *TOND1* confers tolerance to nitrogen deficiency in rice, The Plant Journal, 81: 367-376.
<https://doi.org/10.1111/tpj.12736>

- Zhao Z., He K., Feng Z., Li Y., Chang L., Zhang X., Xu S., Liu J., and Xue J., 2019, Evaluation of yield-based low nitrogen tolerance indices for screening maize (*Zea mays* L.) inbred lines, *Agronomy*, 9(5): 240.
<https://doi.org/10.3390/AGRONOMY9050240>
- Zhou H., Hu J., Yu L., Liu J., Le S., Meng B., Tu H., and Li M., 2025, Screening and evaluation of rice germplasm resources with low nitrogen tolerance, *Acta Agriculturae Universitatis Jiangxiensis*, 47(2): 291-301.
<https://doi.org/10.3724/aauj.2025026>

**Disclaimer/Publisher's Note**

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
