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Transgenic Improvement of Nitrogen Use Efficiency in Wheat Using Root-Specific Promoters

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Abstract Improving the nitrogen fertilizer utilization efficiency (NUE) of wheat is of great significance for achieving high-yield and low-pollution agriculture. Traditional breeding and fertilization strategies are difficult to achieve precise regulation, while the transgenic expression of key nitrogen metabolism genes driven by root-specific promoters provides a new idea for enhancing the NUE of wheat. This study systematically screened and validated a batch of root-specific promoters (such as OsRCc3 and ZmRCP1), constructed expression vectors of them and candidate highly efficient nitrogen metabolism genes (such as *NRTs*, *GS*, etc.), and evaluated the application potential of key promote-gene combinations under different ecological conditions through field experiments. The feasibility of this strategy in ecological security, environmental friendliness and sustainable agriculture was also explored, emphasizing the value of root-specific regulation in precision breeding. The research results show that under low-nitrogen conditions, these transgenic plants exhibit stronger root structures, higher nitrogen absorption capacity, as well as superior nitrogen fertilizer utilization efficiency and yield performance. This research provides theoretical support and technical foundation for achieving the parallel application of reduced nitrogen fertilizer and high yield, and is expected to promote the targeted utilization of rhizosphere functional genes and the development of transgenic nitrogen efficiency breeding.

Keywords Nitrogen use efficiency (NUE); Root-specific promoter; Genetically modified wheat; Key genes for nitrogen metabolism; Precision breeding

1 Introduction

Whether nitrogen fertilizer is applied or not is a key variable determining whether the yield can be increased. Especially for wheat, nitrogen, as a nutrient, is almost indispensable. However, in reality, giving too much is not necessarily a good thing. Nowadays, a considerable amount of nitrogen fertilizer is applied in the fields, but the proportion that is actually absorbed and utilized by the wheat is not high. Where did the remaining nitrogen go? Some entered groundwater, some volatilized into the air, and some even contributed to soil acidification (Tiong et al., 2021; Jiao et al., 2024). It seems that the more input, the higher the output, but in fact, it may bring a double burden on the ecology and economy. Improving the utilization efficiency of nitrogen fertilizers is clearly not only for the sake of yield, but also for the sustainability of farming.

In dealing with this problem, traditional methods are not useless. Selecting wheat varieties with high "nitrogen-consuming efficiency" through breeding and agronomic measures has indeed played a role. However, problems have also emerged. For instance, some varieties, although having a strong nitrogen absorption capacity, have seen a decline in granular nitrogen content, making it difficult to achieve both yield and quality. Moreover, breeding itself is slow and has a long cycle, which is not suitable for rapid response to changes in the agricultural environment (Peng et al., 2022). Another point that is often overlooked is that these methods can actually do very limited in revealing exactly how nitrogen is absorbed, transported and utilized in wheat. Therefore, some people have begun to attempt to use genetically modified means for precise regulation. Although the idea is new, it is precisely the breakthrough point that deserves close attention at present.

This study focuses on analyzing the use of root-specific promoter driver gene expression to enhance the nitrogen utilization efficiency of wheat through transgenic methods, aiming to optimize the absorption and assimilation of nitrogen by the root system and evaluate the effects of targeted transgenic expression on the growth, nitrogen metabolism and yield of wheat under different nitrogen conditions. The significance of this research lies in providing a new biotechnological approach to reduce nitrogen fertilizer input, increase wheat yield, mitigate environmental impact, and thereby promote sustainable agricultural development.

2 Molecular and Physiological Mechanisms of Nitrogen Uptake and Utilization

2.1 Main nitrogen uptake pathways in wheat roots (nitrate and ammonium ions)

Transport is just one link. After nitrogen is absorbed, it still needs to enter the metabolic process. In wheat, the genes of the NRT and AMT families are respectively responsible for bringing nitrate and ammonium into cells, while glutamine synthase (GS) acts like a processing plant, converting the incoming ammonium into amino acids. Interestingly, GS also has several "models", and isoenzymes like GS2 play different roles in different tissues. In addition to these functional genes, some regulatory factors such as WFZP or TaSYD are also involved, mainly regulating the rhythm of root development and gene expression (Liu et al., 2020; Tiong et al., 2021; Kaur et al., 2022; Yao et al., 2025. On the surface, it seems that only the root is changing, but in fact, behind it lies a complex regulatory network in operation.

2.2 Key genes related to nitrogen metabolism

Some key genes in wheat regulate nitrogen absorption and assimilation, including the nitrate transporter gene (NRT), the ammonium transporter gene (AMT), and enzymes involved in nitrogen metabolism, such as glutamine synthase (GS). The NRT gene family, such as NRT1 and NRT2, mediates the absorption and transport of nitrate, while the AMT gene promotes the absorption of ammonium. Glutamine synthase (GS) plays a core role in the process of assimilating ammonium into amino acids, and different isoenzymes, such as GS2, are crucial in the nitrogen assimilation pathway. Transcription factors and chromatin remodeling factors, such as WFZP and TaSYD, regulate the expression of these nitrogen-related genes and coordinate root development and nitrogen absorption efficiency (Liu et al., 2020; Tiong et al., 2021; Kaur et al., 2022; Yao et al., 2025).

2.3 Evaluation indicators and phenotypic traits for nitrogen use efficiency (NUE)

When it comes to evaluating NUE, there is actually no universal standard. Common phenotypic data such as root length, root surface area, number of lateral roots, and even the biomass and grain yield of the aboveground part can all provide clues. If we look at it in more detail, we also need to examine the absorption efficiency of nitrogen, the assimilation rate, and the distribution of metabolic products. Some people will also rely on molecular markers, such as monitoring the expression levels of key genes like NRT and GS, as an indirect judgment method (Figure 1) (Wang et al., 2024; Du et al., 2025; Govta et al., 2025). However, no matter what method is used, the performance of high nitrogen efficiency mostly cannot do without a common point: only when the root system is strong enough and the metabolism is fast enough can stable output be maintained under low-nitrogen conditions.

3 Screening and Characteristics of Root-Specific Promoters

3.1 Common root-specific promoters (e.g., RCc3, OsRSP, AtML1): origin and function

In plant genetic engineering, promoters that can express target genes only in the roots are actually not uncommon. Examples like RCc3 in rice, OsRSP or AtML1 in Arabidopsis thaliana are several that have been studied quite frequently. They are widely used not only because their expression is concentrated in the roots, but also because they are of natural origin - these promoters originally come from some genes that are mainly active in the roots and are often related to root development or nutrient transport. Interestingly, the OsAER1 promoter is not only concentrated in expression but also controlled by a bunch of cis-acting elements. This combination enhances its regulatory effect. In addition, root-specific promoters such as p8463 of corn have long been discovered through genomic mining and their "specificity" has been verified in experiments (Huang et al., 2015; Apriana et al., 2019; Li et al., 2019). Limiting the expression region of transgenic organisms through these promoters can reduce the interference to the above-ground parts, making research and application more targeted.

3.2 Methods for promoter activity identification (e.g., GUS reporter gene, fluorescent markers)

To verify whether a promoter is truly "expressed only in the root", one cannot rely on guessing. A common approach is to carry the reporter gene and work together. For instance, GUS or GFP can be attached behind the candidate promoters, and then tissue staining or in vivo observation can be conducted to determine exactly where they light up in the plant. GUS can display tissue localization, while fluorescent proteins can track expression dynamics in real time. This type of method has been applied to many crops - there are cases in rice, corn, tomato, tobacco, etc. For instance, SlREO and TIP2 have demonstrated stable expression activity in roots (Cui et al., 2020; James et al., 2022; Jenny et al., 2024). Of course, if one wants to further confirm the intensity and specificity, it is still necessary to rely on quantitative methods such as qRT-PCR to make up for it and ensure that the data is more convincing.

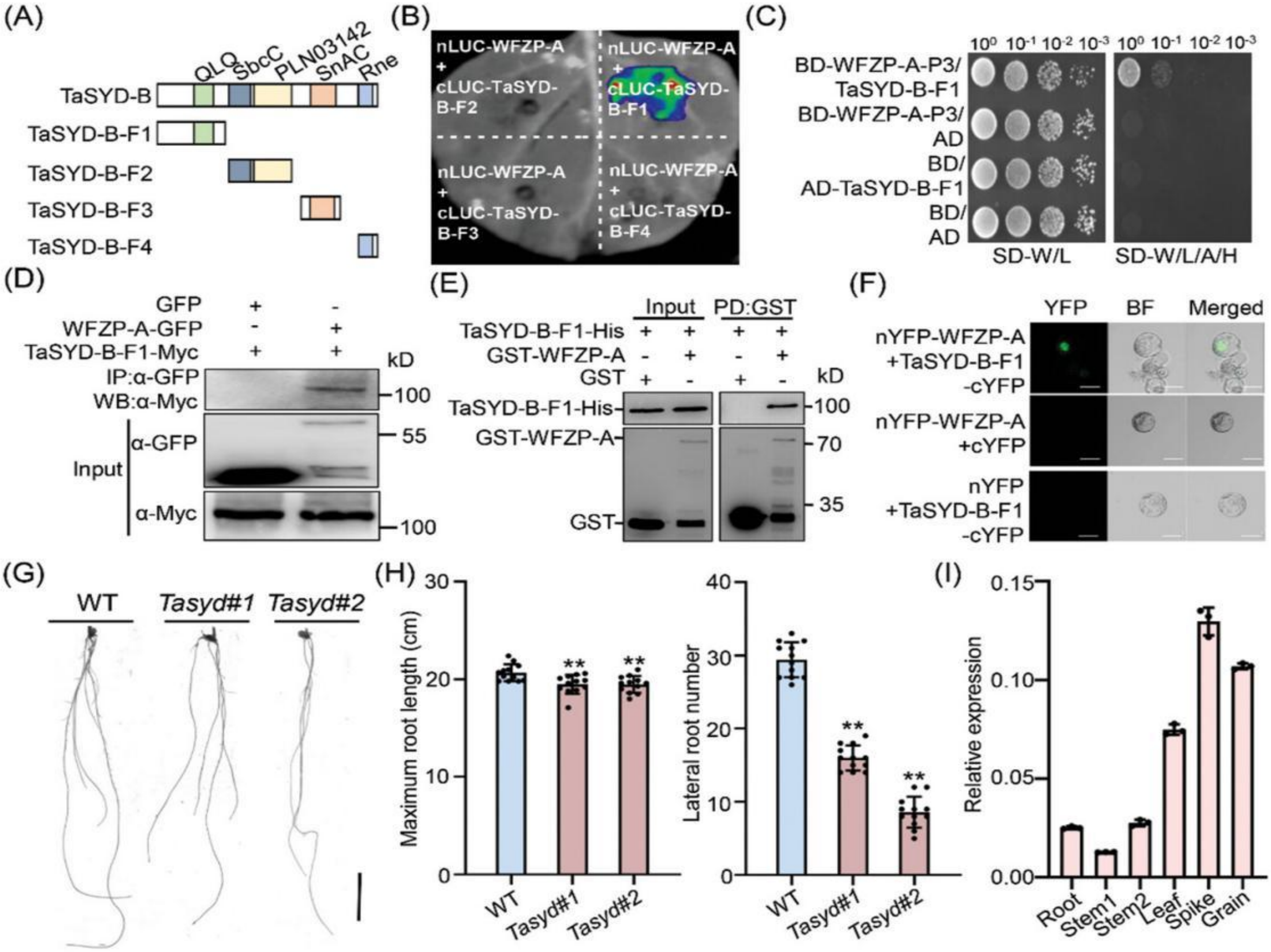

Figure 1 TaSYD-B directly interacts with WFZP-A (Adopted from Du et al., 2025)

Image caption: (A) Schematic diagram showing TaSYD-B and different truncated protein fragments. QLQ, SbcC, PLN03142, SnAC and Rne represent distinct domains. (B) Firefly luciferase (LUC) complementation imaging (LCI) assay showing that TaSYD-B-F1 interacts with WFZP-A in *N. benthamiana* leaves. (C) Yeast two-hybrid (Y2H) showing the interaction between WFZP-A-P3 and TaSYD-B-F1. The transformed yeast cells were grown on synthetic dextrose (SD) medium lacking Trp and Leu (SD-L/W), and selected on SD lacking Trp, Leu, Ade and His (SD-L/W/H/A). AD, pGADT7 vector; BD, PGBKT7 vector. (D) Co-IP assay showing that the TaSYD-B-F1 associates with WFZP-A in *N. benthamiana* leaves (Adopted from Du et al., 2025)

3.3 Expression specificity in different tissues/developmental stages

The "specificity" of root-specific promoters is not absolute and is not equally stable at all developmental stages. Some promoters, such as OsAER1, even if mainly expressed in roots, occasionally show a low level of activity in reproductive sites like anthers or seeds. And the MtPT1 and MtPT2 promoters from alfalfa do not perform exactly the same in different root cell types and are also regulated by nutrient supply. To enhance the specificity of expression, some studies have simply designed synthetic promoters, such as SRSP. By splicing multiple root-specific cis elements, it achieves almost only "exerting force" in the root, with little response elsewhere (Apriana et al., 2019; Cui et al., 2020; Masura et al., 2024). These differences and details must all be taken into account in advance when improving the nitrogen absorption capacity of wheat.

4 Transgenic Construction Strategies for Nitrogen Efficiency Genes

4.1 Selection of candidate genes (e.g., GS1, *NRT1.1*, *NUE1*)

Candidate genes for improving nitrogen use efficiency (NUE) in wheat are usually selected based on their roles in nitrogen absorption, assimilation and metabolism. Key genes include glutamine synthase isoenzymes, such as GS1 and GS2, which catalyze the assimilation of ammonium into amino acids, thereby enhancing the nitrogen cycle within plants. Nitrate transporter genes, such as *NRT1.1*, promote the absorption of nitrate in soil and directly affect the efficiency of nitrogen acquisition. Other promising targets include *NUE1* and amino acid transporter genes, such as *AAP1*, which contribute to the transport and reuse of nitrogen. These genes have been proven to have positive effects on nitrogen fertilizer utilization and yield of various crops such as rice and corn. Therefore, they are strong candidate genes for transgenic improvement of wheat (Chen et al., 2020; Lal et al., 2023; Zhu et al., 2025).

4.2 Construction of expression vectors driven by root-specific promoters

To achieve targeted improvement in nitrogen fertilizer utilization efficiency, researchers fused selected nitrogen-related genes with root-specific promoters to construct expression vectors, ensuring that transgenic expression is confined to the root tissues where nitrogen absorption occurs. For instance, promoters such as OsNAR2.1 and OsAnt1 have been successfully applied to drive the preferential expression of rice roots, thereby enhancing the efficiency of nitrogen absorption and assimilation. The use of root-specific promoters can minimize the potential negative impact on non-target tissues and optimize resource allocation. Expression vectors typically contain regulatory elements for stabilizing high-level expression and can integrate reporter genes to monitor transgenic activity. This strategy can achieve precise spatial control of gene expression, thereby enhancing the effectiveness of transgenic methods in improving nitrogen fertilizer utilization efficiency (Chen et al., 2017; Sisharmini et al., 2019; Tiong et al., 2021).

4.3 Genetic transformation and regeneration process of transgenic wheat

The genetic transformation of wheat is usually carried out by Agrobacterium-mediated method or gene gun method, introducing expression vectors into embryonic callus or immature embryos. After transformation, complete plants were regenerated from the transformed cells using tissue culture techniques and screened on antibiotic or herbicide culture media to identify successful transforms (Han et al., 2025). Molecular analyses were conducted on the regenerated plants, such as PCR, Southern blotting and expression analysis, to confirm the integration and expression of the transgenes. Screen subsequent generations to ensure the genetic stability and heritability of genetically modified organisms. Optimizing transformation efficiency and regeneration schemes is crucial for the production of transgenic wheat lines driven by root-specific promoters with higher nitrogen utilization efficiency (Zhu et al., 2025).

5 Phenotypic and Molecular Analysis of Transgenic Wheat

5.1 Molecular validation (PCR, qRT-PCR, Southern blot)

To confirm whether a genetically modified wheat strain is truly successful, one must first start from the molecular level. The most fundamental approach is to use PCR to detect whether transgenic fragments have integrated into the genome. Sometimes, PCR alone is not enough; Southern blot is also needed to see how many copies have been transferred and where they are inserted. qRT-PCR is also indispensable. It can measure the expression levels of these genes in different tissues, especially paying attention to whether the roots have been successfully activated by the promoter. As Zameer et al. (2025) mentioned, many wheat transgenic materials have copy numbers ranging from 1 to 5, can be stably inherited, and follow Mendelian segregation rules. Although these verification methods are conventional, they are crucial for the subsequent assessment of traits.

5.2 Analysis of root structure, nitrogen uptake ability, and related enzyme activities

Can any changes be observed in appearance after genetic modification? Phenotypic testing often focuses on the root. Especially when materials expressing nitrogen-related genes are driven by root-specific promoters, it is generally observed that the roots are more developed and the biomass is larger. This kind of phenomenon is often associated with the nitrogen absorption capacity. However, merely looking at the root length is not enough; the key is to keep up with the activity of metabolic enzymes, such as glutamine synthase and alanine aminotransferase. Once the enzyme activity increases, it indicates that the nitrogen assimilation function of the root system is also more active. This point has been supported in the studies of Dweikat & Clemente (2017) and Pena et al. (2017), and Zameer et al. (2025) also emphasized the positive impact of this physiological change on plants' tolerance to low-nitrogen environments.

5.3 Field trials: yield, nitrogen recovery efficiency, and NUE comparison

No matter how well genetically modified materials perform in the laboratory, they eventually have to be tested in the fields. In multiple studies, wheat materials that expressed nitrogen metabolism-related genes in their roots demonstrated higher grain yield and nitrogen recovery efficiency than the control group. Materials with the introduction of barley alanine aminotransferase even improved overall nitrogen use efficiency and biomass under field conditions (Pena et al., 2017). However, things are not always linear. Sometimes, environmental factors such as climate, soil, and management methods can devalue these phenotypic results. Therefore, as Chachar et al. (2022) suggested, multi-point and repetitive field trials have become a necessary step to determine whether this material is worth promoting or not.

6 Case Studies

6.1 Study on improving nitrogen uptake efficiency using OsRCc3 promoter to drive TaNRT2.1

To discuss the improvement of nitrogen absorption efficiency in wheat, it is advisable to start with the experience of rice. Some studies have used OsRCc3, a root-specific promoter, to drive the expression of TaNRT2.1. Although there is not much direct data in wheat in this regard, this cross-species gene combination is not a new attempt. When rice nitrate transporters like Os*NRT1.1*A are overexpressed in wheat, they show remarkable adaptability. Especially in a low-nitrogen environment, the root system grows better, the absorption efficiency increases, and the grain yield naturally rises (Wang et al., 2024). From this perspective, if promoters like OsRCc3 can precisely regulate the expression of TaNRT2.1 in the roots, they might be able to open up a new situation in nitrogen utilization efficiency.

6.2 Field evaluation of TaGS1 transgenic wheat constructed with ZmRCP1 promoter

The root promoter ZmRCP1, derived from corn, is used to drive TaGS1 expression, with the aim of enhancing nitrogen assimilation efficiency. Ultimately, the key to TaGS1, the glutamine synthase, lies in whether it is efficient in treating ammonium. Although related field experiments are not overwhelming, existing studies have shown that this root-targeted expression strategy can indeed increase enzyme activity and thereby drive the increase of biomass (Tiong et al., 2021; Zhang et al., 2021). Although the specific field performance data of ZmRCP1+TaGS1 is not yet abundant, from the trend perspective, it is worth delving deeper to achieve higher output without increasing nitrogen input.

6.3 Performance analysis of transgenic wheat under low-nitrogen conditions by the Chinese academy of agricultural sciences

What should be done in a low-nitrogen environment? A series of experiments conducted by the Chinese Academy of Agricultural Sciences have provided their answer. Some schemes are traditional transgenic pathways, such as introducing Os*NRT1.1*A into wheat to ensure it has long roots and well-fed seeds even in nitrogen-deficient fields. Some have employed more "clean" gene editing techniques, such as knockout of TaARE1, which have also achieved the goals of delaying aging and increasing yield (Figure 2) (Zhang et al., 2021; Wang et al., 2024). Interestingly, no matter which technical route it is, the thinking points to a consensus: the root is the key to regulating nitrogen utilization efficiency. The future direction of improvement is likely to focus on the roots rather than merely solving the problem by increasing fertilization.

7 Environmental Impact and Biosafety Assessment

7.1 Potential effects of exogenous genes on non-target tissues and ecosystems

Although the use of root-specific promoters is aimed at confining the expression of exogenous genes to the underground part as much as possible, this strategy cannot completely eliminate the possible concerns about the ecosystem. Could the above-ground tissues "mistakenly trigger" the expression mechanism? This is a question that many people are concerned about. From the experimental results, it can be seen that the expression range of this type of promoter is indeed relatively limited, and the risk of off-target is relatively small. But things don't end there. Even if the effect of genetically modified wheat on rhizosphere soil microorganisms is not obvious, the long-term changes of the ecosystem still need to be observed (Lebedev et al., 2022). And will genes be transmitted to wild relatives through pollen? Or will it have subtle but cumulative effects on non-target organisms? These issues cannot be simply attributed to "low risk" and thus be approved. Continuous ecological monitoring and management remain necessary.

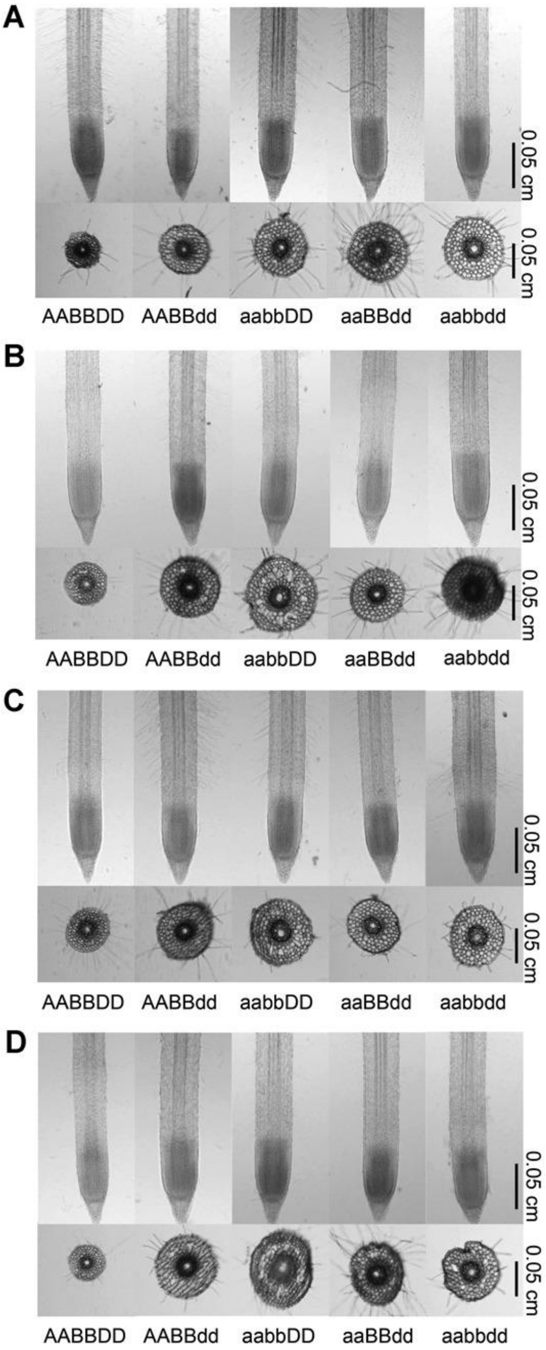

Figure 2 Morphologies of root tips and root cross sections of wild-type and different *taare1* mutant lines (Adopted from Zhang et al., 2021)

7.2 Positive impact of nitrogen fertilizer reduction on environmental pollution control

It is almost common sense in agriculture that the more nitrogen fertilizer is input, the higher the possibility of pollution. But if all the measures are to be cut, what farmers are most worried about is the reduction in production. The issue that genetically modified wheat needs to address is precisely how to reduce nitrogen input while maintaining stable production. Some test strains show promising prospects: under the condition of reduced fertilizer application, the nitrogen absorption efficiency has increased, the yield has not decreased, and the loss of nitrogen in the environment has also been reduced. Long-standing problems such as nitrate leaching, ammonia volatilization and even greenhouse gas emissions have thus been alleviated. This strategy of regulating nitrogen metabolism through root systems does indeed provide a new idea for environmental stress reduction (Tsatsakis et al., 2017). However, whether this effect can be stable in the long term still depends on the actual performance after the field promotion.

7.3 Regulatory and public acceptance of commercial application of transgenic wheat

Technical feasibility does not mean that the market will accept it smoothly. In the process of promoting the commercialization of genetically modified wheat, regulatory standards and public attitudes are often two major obstacles that cannot be avoided. Even though relatively strict biosafety assessment systems have been established in some countries, such as China, where multiple layers of checks are carried out at the environmental and food levels, public doubts still exis (Yu, 2025). Whether one can eat with peace of mind or not, and whether it will affect the ecology, many people are not ignorant of the technology, but rather reluctant to believe it easily. Therefore, relying solely on technical argumentation is not enough. The transparency of regulation is as important as the sincerity of communication. The scientific community can keep saying "safe", but whether it can ultimately gain wide trust still depends on continuous data accumulation and convincing social practice to answer.

8 Conclusion and Future Perspectives

Not all methods to improve nitrogen fertilizer utilization efficiency (NUE) have to address the root cause. Sometimes, focusing on the root system can be more effective. In the study, when nitrate transporters like Os*NRT1.1*A were preferentially overexpressed in the root system, wheat could still absorb sufficient nitrogen under low-nitrogen conditions. Not only did it mature earlier, but its yield also increased. Even better, it causes no disturbance to the growth of the above-ground parts. This type of root-specific promoter does not pursue "omnipotence", but can raise the efficiency of nitrogen absorption and assimilation to a new level. Compared with those one-size-fits-all breeding methods, it is obviously more precise and saves more fertilizer input, which has practical significance for achieving sustainable production.

Of course, reality is never simple. Even if the data is good, there is still a long way to go to widely apply this technology to actual production. First of all, NUE itself is not determined by a single gene. It is influenced by many genetic and environmental factors together, and it is not easy to clearly distinguish the effect. Moreover, wheat itself is difficult to transform, let alone ensure the stable expression of genetically modified organisms. The problems that may arise from off-target effects, coupled with the uncertainties at the policy level, are also obstacles. What's more complicated is that if one wants to superimpose multiple genes to control multiple links of NUE, more complex tools are still needed. A slight mistake may cause side effects.

To move forward, one might need to change their perspective. For instance, by binding the root-specific promoter with CRISPR/Cas9 technology, the regulation of genes can be made more precise. The combined expression (stacking) of different genes related to nitrogen utilization, if operated properly, has the potential to bring about synergistic effects. In addition, transgenic techniques do not have to be used alone. They can be fully combined with high-throughput phenotypic and multi-omics integration breeding strategies to accelerate the screening of nitrogen-saving and high-yield wheat varieties. Finally, apart from technology, public acceptance and environmental safety issues should not be overlooked either. If these achievements cannot be recognized by both farmers and consumers, they may be stuck in the laboratory and unable to leave the "ivory tower".

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Conflict of Interest Disclosure

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

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