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Study on the Physiological Basis of Efficient Nitrogen Utilization and Green Fertilization Strategy of Rice

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Abstract In the context of the current green transformation of agriculture and sustainable ecological development, improving the nitrogen use efficiency of rice has become an important issue to ensure food security and reduce environmental pollution. This study systematically explored the key physiological mechanisms of rice in the process of nitrogen absorption, transport and metabolism, focusing on the expression and regulatory role of key genes such as *OsNRT1.1B*, *OsAMT1.2*, and *OsGS1;1*, as well as the functions of transcription factors such as NLP, DOF, and MYB in the regulation of nitrogen metabolism. Combined with the development trend of green agriculture in recent years, this study further evaluated the practical effects of the "one base and one topdressing" fertilization mode of controlled-release fertilizers and the integrated management with green control technology, and analyzed the synergistic effect of high-efficiency varieties and green fertilization modes through a typical case of the demonstration field in Dahao Village, Jiashan. The study showed that *indica-japonica* hybrid rice and excellent late *japonica* rice varieties have a strong responsiveness to nitrogen supply, and can significantly improve nitrogen use efficiency and yield stability under reasonable cultivation management and precision fertilization. This study not only provides theoretical support and practical path for reducing nitrogen fertilizer and increasing its efficiency, but also provides a reference for the construction of regional rice ecological planting system.

Keywords Rice; Nitrogen use efficiency; Green fertilization; Physiological mechanism; Molecular regulation

1 Introduction

Nitrogen is an essential macronutrient for rice growth, and nitrogen fertilizer application plays an important role in increasing rice yields. However, the nitrogen utilization rate of chemical fertilizers in my country is far lower than that of developed countries, and the utilization rate of nitrogen fertilizer in rice fields in the current season is only about 30%-35% (Yuan et al., 2017). Excessive nitrogen application leads to insufficient nitrogen absorption by crops through leaching, volatilization and runoff loss, causing environmental problems such as soil acidification, groundwater nitrate pollution and water eutrophication. At the same time, agriculture has become an important source of greenhouse gas emissions such as nitrous oxide. Excessive use of nitrogen fertilizers can also cause rice to grow too fast, induce an increase in the risk of pests and diseases, thereby increasing the amount of pesticide application and forming a vicious circle. Therefore, improving the utilization efficiency of nitrogen fertilizers and reducing excessive nitrogen fertilizer input have become urgent needs for the current sustainable development of agriculture.

My country is a major rice producer and consumer, and rice yield is related to national food security. In the case of limited arable land, achieving high and stable yields must rely on reasonable fertilization. However, the long-standing concept of "more is better" among farmers has led to high nitrogen fertilizer input intensity, decreased fertilizer utilization efficiency, and diminishing yield benefits (Park et al., 2023). According to statistics, the nitrogen fertilizer utilization rate of rice in my country was about 60% in the 1980s, and it dropped to about 30% in the 21st century (Ju et al., 2024). Improving fertilization methods and cultivating nitrogen-efficient varieties to achieve "less fertilizer and higher yield" have become key strategies for the sustainable development of rice farming. Improving rice NUE has multiple meanings: for the environment, it can reduce fertilizer use and nitrogen emissions, alleviate agricultural non-point source pollution and greenhouse gas emissions; for the

economy, it can reduce grain production costs and improve fertilizer utilization efficiency; for production, it can achieve "higher output with less input" by increasing the output per unit of nitrogen fertilizer, and ensure grain production capacity and farmers' income.

This study will systematically explain the physiological mechanism and implementation path of rice nitrogen efficient utilization, analyze the absorption, transport and redistribution process of rice, and the relationship between nitrogen metabolism and plant growth and development; summarize the functions and regulation of genes related to rice nitrogen efficient utilization identified in recent years, including key transporters, assimilation enzymes and transcription factor networks; explore the green fertilization technology for controlling nitrogen supply and reducing environmental impact, and its application effect in field trials and large-scale demonstrations; compare the response differences of different types of rice varieties (such as *indica-japonica* hybrid rice and conventional *japonica* rice) to nitrogen fertilizer and their compatibility with fertilization strategies; through the case of Jiashan area, introduce the practical model and results of the synergistic effect of green fertilization technology and high-efficiency varieties. This study summarizes the core mechanism of rice nitrogen efficient utilization and the value of green fertilization in sustainable agriculture, and looks forward to future research directions and promotion and application, providing a theoretical basis and practical reference for rice fertilizer saving and efficiency improvement.

2 Physiological Mechanisms of Nitrogen Uptake and Transport in Rice

2.1 Root mechanisms for nitrogen uptake

Rice roots are the main organs for nitrogen absorption, and their nitrogen absorption efficiency is affected by factors such as root morphology, physiological activity, and soil nitrogen supply. In rice soil, nitrogen mainly exists in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Rice prefers to absorb ammonium nitrogen. High-affinity and low-affinity ammonium transporters (such as AMT) are distributed on the epidermal and cortical cell membranes of the roots, which can actively absorb NH_4^+ in the soil solution. At the same time, due to the release of oxygen by the aerenchyma of the rice roots, local nitrification occurs in the rhizosphere, and rice also has a strong absorption capacity for NO_3^- (Wang et al., 2020). There are two major types of nitrate transporters in the root system, NRT1/NPF and NRT2. Among them, the NPF family is responsible for the low-affinity uptake of large amounts of NO_3^- , and the NRT2 family is responsible for the high-affinity uptake of NO_3^- (Lee, 2021). For example, OsNRT2.3b and OsNRT2.3a are located in the phloem and xylem, respectively, and mediate the upward transport of nitrogen from the roots to the stems and leaves. Another important pathway is the direct absorption of organic nitrogen by the roots, such as amino acids, which enter the root cells through specific carrier proteins. Rhizosphere microorganisms can increase the effective nitrogen supply in the soil through nitrogen fixation and ammonification. Inoculation of nitrogen-fixing bacteria can enhance the supply of NH_4^+ in the rhizosphere and increase the nitrogen uptake of rice (Wang et al., 2024). Nitrogen uptake by rice roots is the result of the combined action of multiple membrane transport proteins and rhizosphere biological processes, and its efficiency determines the nitrogen nutritional status and subsequent utilization efficiency of the plant.

2.2 Transport and redistribution of nitrogen within the plant

The nitrogen absorbed by the rice roots needs to be effectively distributed in the body to maximize its use for growth and yield formation. The NH_4^+ absorbed by the roots is assimilated into glutamine in the roots through the glutamine synthetase-glutamate synthetase (GS-GOGAT) pathway, and then converted into other amino acids, which are transported upward to the stems and leaves through the xylem sap flow. Part of NO_3^- can be reduced to nitrite by nitrate reductase (NR) in the roots and then further assimilated by the leaves; unreduced NO_3^- enters the leaves with the transpiration flow and is assimilated into organic nitrogen in the leaves. The redistribution of nitrogen between various organs of the plant is crucial for the late filling of rice. After heading, a large amount of protein stored in the nutrient leaves and stem sheaths is decomposed to produce amino acids, which are transported to the grains through the phloem for protein synthesis. It is estimated that about 70%-80% of the nitrogen in rice grains comes from the retransportation of nutrient organs (Padhan et al., 2023). Efficient nitrogen reuse depends on the participation of amino acid transporters, such as OsNRT2.3b and OsNPF7.2 located in the phloem, which play an important role in nitrogen redistribution. Nitrogen redistribution efficiency (nitrogen

harvest index) is affected by the source-sink characteristics of the variety: nitrogen-efficient genotypes often have greater late assimilation capacity and slower leaf senescence rate, which significantly increases the dry matter and nitrogen accumulation after heading. For example, nitrogen-efficient and high-yield varieties still maintain a high leaf area index and green functional leaves at maturity, and have more nitrogen assimilated later to supply grains than ordinary varieties. The transportation and redistribution process of nitrogen in rice plants between sources (assimilation organs such as leaves) and sinks (storage organs such as grains) is complex and efficient, and is a key factor in determining the final yield and quality.

2.3 Synergistic relationship between nitrogen metabolism and growth regulation

The nitrogen metabolism process of rice is closely coupled with carbohydrate metabolism and growth and development. Nitrogen assimilation requires a large amount of adenosine triphosphate (ATP) and reducing power, and its energy and carbon skeleton come from carbon metabolites provided by photosynthesis and respiration. At the same time, the nitrogen supply level will significantly affect the carbon allocation of plants: sufficient nitrogen promotes tillering and leaf growth, and increases the proportion of photosynthetic products used to build assimilation products such as proteins; while under nitrogen limitation, plants tend to reduce new leaf growth and increase carbon allocation to the root system to enhance the ability to obtain nitrogen. Studies have shown that maintaining a suitable C/N balance is crucial for normal crop growth and yield formation (Wang et al., 2020). There is a molecular regulatory network in plants that integrates carbon and nitrogen signals. For example, when nitrogen nutrition is sufficient, the increased abundance of amino acids will feedback inhibit certain steps in carbon assimilation, thereby avoiding excessive carbon accumulation; conversely, when nitrogen is deficient, plants enhance the nitrogen absorption capacity of the root system by increasing the distribution of carbohydrates in the roots. Multiple transcription factors are involved in coordinating C-N metabolism, such as Dof and bZIP factors, which sense the levels of carbon/nitrogen metabolites and regulate the expression of downstream enzyme genes to adapt to changes in nutritional status. Overexpression of maize Dof1 transcription factor can improve rice photosynthesis and the flow of carbon to nitrogen assimilation pathways, significantly enhancing plant growth and nitrogen accumulation under low nitrogen conditions. Another example is factors such as OsNAC42, which can simultaneously upregulate nitrate transport and growth-related genes to achieve the effect of maintaining a higher growth rate under low nitrogen. Therefore, nitrogen metabolism and plant growth and development are closely coordinated through a complex signaling network. When optimizing cultivation management, the effects of nitrogen supply on growth processes such as tillering and heading should be comprehensively considered to achieve synchronous coordination of nutrient supply and crop growth requirements (Du et al., 2013).

3 Expression and Regulation of Genes Related to Nitrogen Use Efficiency in Rice

3.1 Role of key genes in nitrogen utilization

With the deepening of rice genome functional research, a series of key genes affecting nitrogen absorption and utilization have been identified. Functional gene mutations in nitrogen transport and assimilation pathways lead to differences in plant NUE. For example, the nitrate transport gene *OsNRT1.1B* has functional differences due to allelic variation between *indica* and *japonica* subspecies: Studies have found that the *indica* rice *OsNRT1.1B* allele increases the root system's NO₃⁻ uptake activity and root-leaf nitrogen transport efficiency, so that the nitrogen absorption and grain yield of the near-isogenic *japonica* rice are significantly increased after the introduction of *indica* rice *OsNRT1.1B* (Lee, 2021). Therefore, *OsNRT1.1B* is considered to be one of the important sites controlling rice nitrogen efficiency (Tang et al., 2019). Similarly, in terms of ammonium nitrogen absorption, the root high-affinity ammonium transporter *OsAMT1.2* plays a key role. Some studies have achieved higher growth and grain yield of rice under low nitrogen conditions by simultaneously enhancing the expression of *OsAMT1.2* and GOGAT enzyme genes. The rate-limiting enzyme genes of nitrogen assimilation metabolism have a significant effect on NUE. The glutamine synthetase gene *OsGS1;1* is mainly expressed in roots and old leaves, responsible for assimilating absorbed or transported NH₄⁺ into glutamine, and is a key enzyme in the nitrogen recycling process. The *OsGS1;1* knockout mutant showed poor growth and decreased nitrogen content in the grain, indicating that this gene is indispensable for nitrogen redistribution and yield formation. However, simply overexpressing nitrogen assimilation enzymes does not necessarily increase NUE. For example, overexpression of

rice *OsGSI;1* has led to an imbalance in carbohydrate distribution and a decrease in yield (Figure 1) (Liu et al., 2022). Therefore, moderate expression and spatiotemporal regulation of key enzyme genes are conducive to improving overall nitrogen efficiency. There are many genes that determine the efficiency of nitrogen absorption, assimilation and recycling in rice, including transporters, metabolic enzymes, etc.

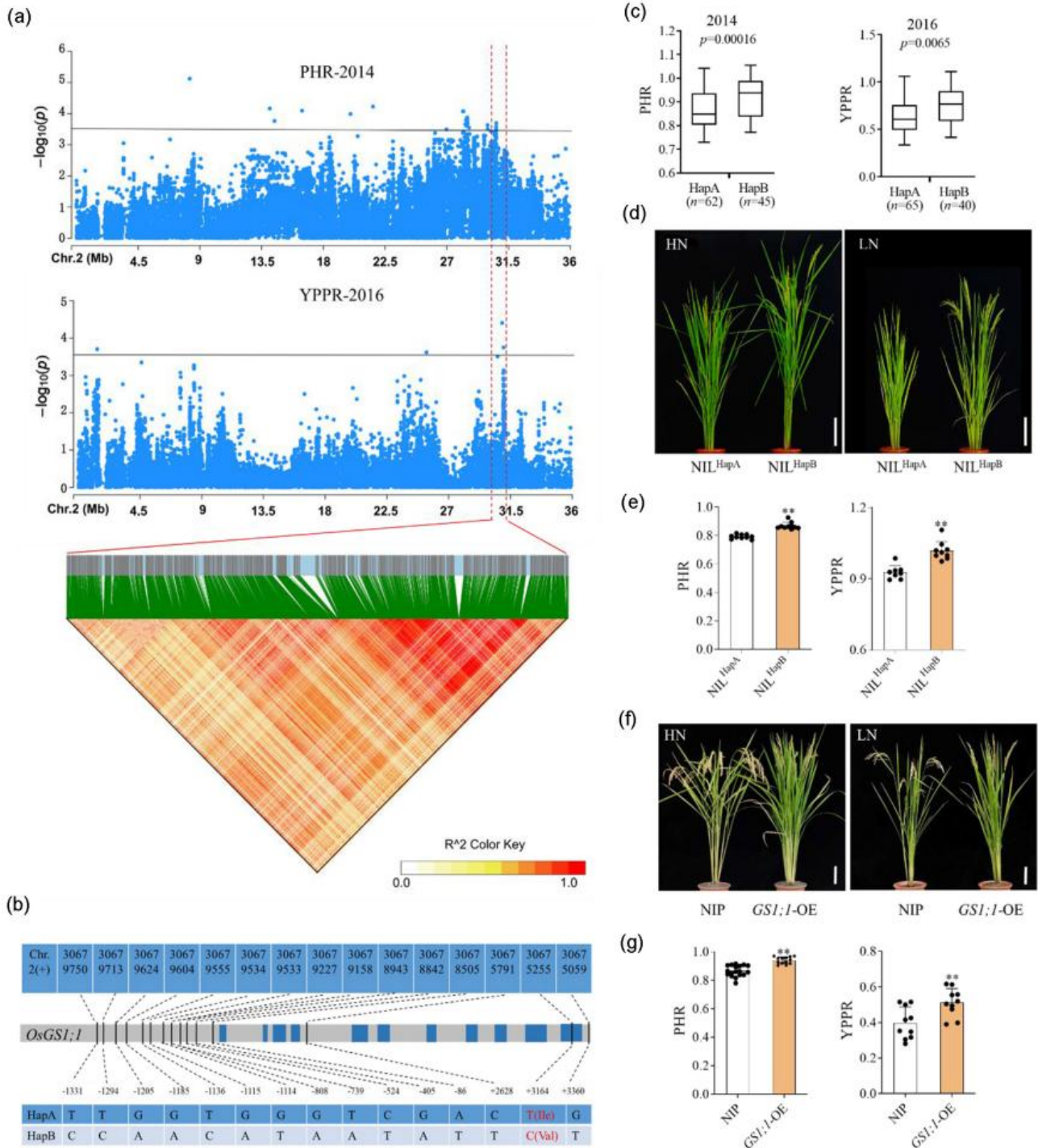


Figure 1 Identification and functional validation of *OsGSI;1* on chromosome 2. (a) Local Manhattan plot (top) and linkage disequilibrium (LD) heatmap (bottom) surrounding the peak on chromosome 2. The red dashed line indicates the candidate region for the peak. (b) Gene structure of *OsGSI;1* and DNA polymorphism in this gene. The gray box represents the promoter region, untranslated regions (UTRs), and introns, and light blue represents coding regions. (c) Comparison between *OsGSI;1* haplotypes. (d-e) The plant height and yield per plant of NILHapA and NILHapB in high nitrogen (HN) and low nitrogen (LN) fields. (f-g) The plant height and yield per plant of wild-type (WT) and overexpression (OE) lines in HN and LN fields. Values are mean \pm SD ($n \geq 10$). Bar = 20 cm. * $P < 0.05$, ** $P < 0.01$ (Adopted from Liu et al., 2022)

3.2 Nitrogen-regulated transcription factors

Plants use a series of transcriptional regulatory factors to sense and respond to external nitrogen levels, thereby coordinating the expression of many functional genes. NIN-like proteins (NLP) are important nitrogen signal transcription factors discovered in recent years. In model plants, AtNLP7 has been shown to regulate the nitrate-responsive gene network. Functional studies of the rice NLP family have shown that OsNLP1 and OsNLP3 are involved in regulating the expression of related genes under both nitrogen-sufficient and nitrogen-deficient conditions. OsNLP1 responds most quickly to nitrogen starvation. When nitrogen is deficient, its active form rapidly translocates into the nucleus, inducing the expression of a series of nitrate transport and assimilation genes, thereby improving the plant's ability to absorb and utilize nitrogen. Rice overexpressing OsNLP1 significantly increased grain yield under low-nitrogen field conditions, and maintained higher nitrogen accumulation and utilization efficiency under high nitrogen conditions (Alfatih et al., 2020; Zhang et al., 2022). OsNLP3 mainly plays a role when nitrate is sufficient, promoting the expression of genes related to nitrogen absorption and assimilation, optimizing plant nitrogen metabolism, and enabling rice to obtain higher yield and NUE under normal nitrogen application conditions. OsNLP4 has been reported to be a key factor in improving rice NUE: field experiments have shown that knocking out OsNLP4 will lead to a significant decrease in rice yield and NUE under different nitrogen application levels, while overexpressing OsNLP4 can increase yield by about 30% and NUE by about 47% under moderate nitrogen application.

In addition to the NLP family, DOF and MYB transcription factors are also involved in efficient nitrogen regulation. Some members of the DOF (DNA binding and one finger protein) family can affect the coordination of carbon-nitrogen metabolism. For example, exogenous introduction of maize ZmDof1 can enhance photosynthesis and assimilation during the grain filling period of rice, significantly improving growth and nitrogen accumulation under low nitrogen conditions. Rice's own DOF factor OsDof substances (such as OsRDD1) have also been shown to play a role in nitrogen absorption and assimilation. Their overexpression can promote the root system's absorption of ammonium ions and other nutrients and accumulate more assimilates. In terms of MYB transcription factors, OsMYB305 is a transcriptional activator that senses nitrogen in roots. OsMYB305 is upregulated when nitrogen is deficient, and overexpression of this gene significantly increases the number of tillers, aboveground biomass, and nitrogen content of rice plants under low nitrogen conditions. Mechanistically, OsMYB305 activates the expression of high-affinity nitrate transport system-related genes (such as OsNRT2.1/2.2, OsNAR2.1) and nitrogen assimilation enzyme genes, increases the root system's uptake rate of nitrate nitrogen, and inhibits root lignin synthesis-related genes to allow more carbohydrates to be used for nitrogen assimilation (Wang et al., 2020). The activities of key enzymes such as nitrate reductase (NiR) and GOGAT in the roots of its overexpression strains were significantly enhanced, promoting nitrogen assimilation and plant growth. A series of transcription factors such as NLP, DOF, and MYB regulate the nitrogen response network through different pathways: some directly bind to nitrate response elements to activate downstream genes (such as NLP); some indirectly improve nitrogen utilization by affecting carbon metabolism and hormone pathways (such as DOF and MYB). The discovery of these factors provides new molecular targets for improving the intrinsic nitrogen efficiency of crops.

3.3 Application of molecular breeding and gene editing technologies to improve nitrogen efficiency

Based on the understanding of nitrogen efficiency-related genes, molecular breeding has opened up a new path for breeding nitrogen-efficient rice. On the one hand, quantitative trait loci (QTL) positioning and association analysis have identified many genes that affect NUE. For example, a genome-wide association study found that there is a "hotspot" on chromosome 1 that is significantly enriched in NUE-related genes, among which an excellent haplotype of OsNPF6.1 can improve nitrogen absorption and yield under low nitrogen conditions. According to statistics, more than 1,000 genes related to NUE have been reported, including a large number of transporters and transcription factors. By integrating multi-omics data, the range of candidate genes can be further narrowed and key control genes can be locked. These genetic resources provide rich materials for the breeding of nitrogen-efficient varieties. On the other hand, gene editing technology (such as CRISPR/Cas9) has shown great potential in improving crop NUE. Site-specific editing of existing NUE alleles in rice can quickly create ideal

genotypes. For example, by replacing the key nucleotides of *japonica* rice *OsNRT1.1B* with indica rice types through base editing, a new strain with stronger nitrogen absorption capacity was successfully cultivated, and the yield was significantly improved under low nitrogen fertilizer supply (Liu et al., 2023). For another example, a study targeted the regulation of a gene *DNR1* that affects auxin balance and obtained high-NUE and high-yield materials. Aggregating multiple favorable sites is also an effective strategy. Gene editing can be used to simultaneously knock out negative regulatory genes and introduce excellent alleles in a variety, thereby improving nitrogen efficiency in all aspects. Some scholars have proposed a "gene pyramid" scheme, combining favorable genes for different links such as nitrate nitrogen absorption, ammonium assimilation and growth regulation. At present, emerging technologies for improving rice nitrogen efficiency also include genomic selection and epigenetic regulation. Molecular breeding and biotechnology are accelerating the breeding process of nitrogen-efficient rice. In the future, through the precise improvement of key genes and the aggregation of multiple genes, it is expected that new rice varieties with high and stable yields under low input conditions will be cultivated.

4 Exploration of Green Fertilization Management Technologies in Field Applications

4.1 Optimization and configuration of controlled-release fertilizer technologies

Traditional nitrogen application methods such as one-time basal application or multiple broadcasting are prone to cause excessive nitrogen supply in the early stage, insufficient supply in the later stage, and serious nutrient loss. Controlled-release fertilizer (CRF) delays nitrogen release through coating or chemical inhibitors, which can achieve fertilizer supply in synchronization with crop nutrient demand. The application of slow-release fertilizer on rice can reduce the number of applications of tillering fertilizer and panicle fertilizer and even achieve the effect of "one-time fertilization". Studies have shown that compared with multiple applications of traditional urea, one-time basal application of CRF can increase rice yield by about 15%-30%, and significantly improve nitrogen fertilizer partial productivity and nitrogen recovery rate. Gil-Ortiz et al. (2020) reported that in rice field experiments, controlled-release nitrogen fertilizer treatment increased yield by about 35% compared with conventional fertilization, and increased the nitrogen content and other nutrient content of plant leaves. A large number of domestic experiments have also confirmed that slow-release fertilizers can increase rice yield and nitrogen fertilizer utilization efficiency. For example, experiments have shown that a one-time application of a formula fertilizer containing slow-release nitrogen can increase yield by 8% compared with applying urea in batches. Another example is that Xu (2021)'s research shows that the application of controlled-release fertilizers combined with conventional nitrogen fertilizers can significantly increase the nitrogen absorption and nitrogen utilization rate of rice plants. In demonstrations in Jiashan and other places, controlled-release fertilizers with different release rates were scientifically proportioned with quick-acting nitrogen fertilizers, and mechanical side deep fertilization technology was used to achieve a one-time concentrated application of base fertilizer and tillering fertilizer, so that rice can still obtain sufficient nutrient supply in the middle and late stages of growth. Compared with the traditional "three fertilizers and two topdressing", this model greatly reduces the number of fertilizations and labor inputs, while significantly increasing production and efficiency. Therefore, the optimized application technology of controlled-release fertilizers is an important way to reduce nitrogen and increase efficiency in rice fields, and can provide strong support for green fertilization.

4.2 Integration with green pest control technologies

While reducing the application of chemical fertilizers, optimizing plant protection technology to achieve the coordination of fertilizer management and pest and disease control is one of the directions for green and efficient rice production. Excessive nitrogen application often leads to the growth of lush plants, which increases the adaptability of pests and diseases, thereby increasing the use of pesticides. Studies have shown that appropriately reducing the use of nitrogen fertilizer can reduce the risk of rice blast, sheath blight, etc., and improve the plant's resistance to lodging (Deng et al., 2023). In order to achieve fertilizer reduction without reducing production or increasing diseases, it is necessary to combine fertilizer management with plant protection technology to formulate a comprehensive management plan. For example, in the demonstration fields in Jiashan, Zhejiang, the "unified fertilizer supply and unified plant protection" model is promoted: slow-release fertilizer is applied deep

on the side during mechanical transplanting, and topdressing is applied in time according to the growth of the field in the later stage, supplemented by planting attracting plants such as cosmos and vetiver and placing sex attractants to kill borers, reducing the dependence on chemical pesticides. At the same time, biological pesticides and biological microbial agents are used to control pests and diseases, improve plant health, and enhance their nutrient utilization efficiency (Ren, 2017; Li, 2024). Other studies have tried models such as rice-fish farming and rice-duck farming to increase breeding income while reducing the occurrence of pests, and found that rice-fish farming can also reduce field methane and nitrous oxide emissions, achieving "killing two birds with one stone". The integration of green prevention and control and scientific fertilization can maintain high rice yields under the condition of nitrogen reduction and reduce environmental and health risks. This collaborative management requires agricultural technicians to accurately monitor crop growth and pest occurrence, dynamically adjust fertilizer and pesticide input according to actual conditions, and achieve reduced and efficient agricultural inputs.

4.3 Field trials for fertilizer reduction and efficiency enhancement, and benefit evaluation

A large number of field experiments have shown that rice yields can be maintained while reducing nitrogen fertilizer inputs by optimizing fertilization. Generally speaking, reducing nitrogen by 10%-20% in high-yield fields has no significant effect on yield, while nitrogen fertilizer utilization efficiency can be significantly improved (Hu et al., 2019). For example, experiments in South China found that compared with farmers' conventional nitrogen application, rice yields in the treatment of 20% nitrogen fertilizer reduction only decreased slightly or remained basically the same, but the rice produced per kilogram of nitrogen fertilizer increased, and the agronomic efficiency of nitrogen fertilizer was improved. High-yield nitrogen and high-efficiency varieties perform particularly well under nitrogen reduction conditions, with significantly higher biomass and yield than general varieties, and higher nitrogen grain production efficiency (Fu et al., 2023). This shows that through the combination of varieties and fertilization measures, "reducing nitrogen without reducing production" can be achieved. Nitrogen fertilizer reduction also brings environmental and economic benefits. For example, a long-term positioning study showed that compared with the usual amount, reducing nitrogen by about 15% can reduce the risk of nitrogen leaching in rice fields and improve fertilizer utilization without reducing production. In the Jiashan demonstration, the application of side deep fertilization technology reduced the amount of nitrogen fertilizer by about 20% compared with traditional broadcasting, while the average yield per mu remained above 600 kg, which reduced the input of nitrogen fertilizer per kilogram of rice and improved the planting efficiency. Of course, the potential for nitrogen reduction also varies by region and production level. Some studies have used models to evaluate the nitrogen reduction potential in different rice-growing areas. For example, in the black soil area of Northeast China, nitrogen can theoretically be reduced by about 17% with little impact on yield (Yin et al., 2022). Field experiments and economic analysis support the conclusion that it is feasible to reduce fertilizer input without significantly affecting rice yield by optimizing fertilization structure and methods. This is also an effective way to increase fertilizer utilization, reduce costs and environmental costs.

5 Nitrogen Response Characteristics of Superior Rice Varieties

5.1 Nitrogen sensitivity of *indica-japonica* hybrid rice

Indica-japonica hybrid rice varieties usually have higher yield potential, but they often show stronger response sensitivity to nitrogen fertilizer levels. Compared with conventional rice, hybrid rice requires slightly less nitrogen to produce 100 kg of rice (about 1.4-2.0 kg), but due to its high yield and large population biomass, the total nitrogen required per hectare is still higher than that of conventional rice. Hybrid rice is also generally more efficient in absorbing and utilizing nitrogen fertilizer. With reasonable cultivation measures, the nitrogen fertilizer recovery rate of high-yield hybrid rice varieties in the season can reach 40%-45%. Taking the widely planted *indica-japonica* hybrid rice "Yongyou" series as an example, its population grows vigorously, with large panicles and many grains, and can fully exert its yield potential under high nitrogen conditions; but at the same time, excessive nitrogen is more likely to lead to its late greed for green, lodging and increased risks of diseases and insect pests, so the amount of nitrogen fertilizer in the late stage needs to be strictly controlled. As a representative variety of this series, "Yongyou 33" has a strong nitrogen response ability. The experiment showed that under sufficient nitrogen application, the number of spikelets in the "Yongyou 33" population far exceeded that of

ordinary *japonica* rice, and the yield advantage was obvious. Under the nitrogen reduction treatment, the decline in its yield was also greater than that of some conventional *japonica* rice. This shows that high-yield hybrid rice such as "Yongyou 33" is more sensitive to nitrogen supply: sufficient nitrogen can maximize its hybrid advantage to achieve high yield, but insufficient nitrogen is more likely to result in small panicles and few grains, and a decline in yield. Therefore, for this type of variety, it is advisable to adopt a fertilization strategy of "promoting in advance and controlling in the later stage", that is, sufficient nitrogen supply in the early stage to promote tillering, appropriate nitrogen control in the later stage to prevent greed, and good water pipes and plant protection to reduce lodging and the occurrence of diseases and insects. Under reasonable management, *indica-japonica* hybrid rice can maintain good nitrogen efficiency at a higher yield level. According to Zhou et al. (2020)'s study of 24 "Yongyou" series varieties, one type of high-yield hybrid rice achieved both high yield and high nitrogen efficiency. Its total nitrogen absorption during maturity was only about 5% higher than that of ordinary types, but its yield was more than 13% higher. These varieties showed higher panicle number, panicle formation rate and late dry matter accumulation, and were able to transport more nitrogen to the panicle. By combining variety selection and fertilization regulation, the high yield potential and high nitrogen efficiency of hybrid rice can be taken into account to achieve "high yield and high efficiency".

5.2 Nitrogen uptake and utilization of conventional late *japonica* rice

Compared with hybrid rice, conventional *japonica* rice varieties have slightly lower yield potential but more stable rice plant types, and their response to nitrogen fertilizer levels is relatively slow. Take two new high-quality late *japonica* varieties in Zhejiang, "Jiahe 567" and "Xiushui 1717", as examples: they can show higher nitrogen absorption and utilization capabilities and yield levels under moderate nitrogen input, and the marginal effect of excessive nitrogen application on yield is not as obvious as that of hybrid rice. According to the demonstration results in Jiashan County, under the conditions of unified fertilization management (pure nitrogen of about 15.4 kg/mu, one base fertilizer + one topdressing), the average per-mu yields of "Jiahe 567" and "Xiushui 1717" were about 620 kg and 600 kg, respectively, which was not much different from the treatment with increased nitrogen fertilizer. This shows that these conventional late *japonica* rice varieties have high nitrogen utilization efficiency and can maintain relatively ideal yields under less nitrogen supply. The mechanism may be that the plant type is more compact and the root system is more active, which can absorb soil nitrogen more fully; at the same time, the panicle structure and fruiting rate are more optimized, and the number of grains produced per unit nitrogen is higher (Huang et al., 2024). For example, Jiahe 567 has moderate tillering and high panicle rate, and the yield difference between the control nitrogen level and the 20% nitrogen reduction treatment is very small. For example, Xiushui 1717 has high-quality rice and strong stems, and still maintains a good fruiting rate and thousand-grain weight when nitrogen fertilizer is low. Conventional late *japonica* rice is more sensitive to excessive nitrogen fertilizer in terms of rice quality: studies have found that excessive nitrogen application will reduce the smoothness and taste of rice, while moderate reduction of nitrogen fertilizer is beneficial to improving the quality of high-quality *japonica* rice (Jiang et al., 2022). Therefore, for high-quality conventional rice such as Jiahe 567 and Xiushui 1717, a reasonable nitrogen fertilizer level should be determined based on the target yield to balance yield and quality. Conventional late *japonica* rice varieties show good nitrogen utilization "resilience" and can achieve a high nitrogen harvest index and fertilizer utilization rate under moderate nitrogen supply. This type of variety is suitable for promotion in nitrogen fertilizer reduction measures, which can reduce fertilizer input without significant loss of yield.

5.3 Compatibility analysis between varieties and fertilization strategies

The optimal fertilization strategies for different rice varieties are different, and the matching (compatibility) of variety characteristics and fertilization measures is the key to achieving high yield and high efficiency. For high-yield hybrid rice, it should be emphasized to "apply nitrogen according to the variety", provide sufficient nitrogen according to its high biomass and large panicle characteristics, but at the same time avoid excessive nitrogen in the later stage. For example, for the Yongyou series of hybrid rice, the basal tillering fertilizer should be relatively sufficient to establish a sufficient number of panicles, and the panicle fertilizer should be appropriate

to ensure that the fruit is not too green. Accordingly, slow-release fertilizer can be used in combination with delayed fertilization to make the nitrogen supply more balanced and meet its "large reservoir capacity" demand for continuous nitrogen supply. On the contrary, for conventional *japonica* rice varieties, due to their relatively flat nitrogen response curve, "nitrogen reduction and dense planting" can be appropriately used to give play to their group advantages. That is, by increasing the basic seedlings or planting density to compensate for the decrease in panicles that may be caused by nitrogen reduction, while stabilizing the number of grains per panicle and grain weight, so that the total yield is not affected (Hu et al., 2019). The amount of nitrogen applied to this type of variety in the later stage should not be too high to avoid lodging and reduce quality. It can be combined with bio-organic fertilizer or green manure to replace part of the chemical fertilizer nitrogen to maintain soil nutrient balance. Variety \times fertilization interaction studies also show that varieties with high nitrogen efficiency genotypes have a more obvious relative advantage under low nitrogen, while nitrogen inefficient genotypes require high fertilizer input to exert their yield potential. Therefore, by screening the yield performance of different varieties under nitrogen reduction conditions through field trials, we can provide a basis for formulating the optimal fertilization plan for each variety. With the development of precision agriculture, the use of drone spectroscopy and other technologies to monitor the nitrogen nutrition status of different varieties in real time and implement differentiated fertilization will further improve the matching degree between varieties and fertilization management. Optimizing fertilization according to variety characteristics can, on the one hand, allow high-yield varieties to "eat enough without waste", and on the other hand, allow high-efficiency varieties to "eat smarter and more efficiently", thereby maximizing the potential of varieties and the benefits of fertilizers at the same time.

6 Case Study: Integrated Practice of Green Fertilization and High-Efficiency Varieties in Jiashan Area

6.1 Promotion path of demonstration field model: "unified fertilizer supply and unified management"

Jiashan, Zhejiang, is located in the grain area of Taihu Plain. In recent years, it has explored a rice production model that integrates green fertilization and centralized management. In the 10 000-acre demonstration base in Dahao Village, Huimin Street, Jiashan, the establishment of a cooperative to implement unified fertilizer supply and unified plant protection management has greatly improved the implementation rate and efficiency of agricultural technical measures (Figure 2). The specific approach is: the agricultural technicians of the cooperative formulate a unified fertilization plan for the village based on soil fertility and target yield, and centrally purchase new fertilizers such as slow-release fertilizers to supply farmers; in terms of pest control, the cooperative organizes the implementation of green prevention and control and unified prevention and control, such as the unified installation of insect traps, the distribution of sex pheromone traps, and the planting of insect-attracting flowers in the field to reduce the use of chemical pesticides. The promotion path of this model in Jiashan is to first demonstrate successfully in the pilot village, and then radiate and drive in the grain functional areas of the county. Dahao Village takes advantage of its own advantages as a pure agricultural village to create a brand of "unified fertilizer supply and unified plant protection", and promotes it to surrounding towns through on-site observation and technical training. By 2024, the cooperative where Dahao Village is located has planted more than 3,500 mu of rice, and all of them have implemented soil testing and formulation and one-time slow-release fertilizer application. The pest control uses high-efficiency and low-toxic pesticides and reduces the dosage by about 20%. This model has achieved the transformation from decentralized operation of farmers to centralized service of cooperatives, making it possible for small farmers to adopt advanced technologies. Through large-scale coordination, it has not only reduced procurement and operation costs, but also ensured the large-scale implementation of green efficiency-enhancing technologies (Wu et al., 2021). Practice has proved that under the "unified fertilizer supply and unified management" model, the labor productivity and resource utilization efficiency of rice production have been significantly improved: the cooperative combines mechanical transplanting, side deep fertilization and unified prevention and control, and the average fertilizer and pesticide costs per mu are reduced by 15% and 10% respectively compared with traditional methods, while the average per mu yield has increased slightly. Jiashan's experience shows that coordinated management led by new business entities in grain-producing areas is an important way to achieve green and efficient production.



Figure 2 Planting base

6.2 Application effect of controlled-release fertilizer “one basal, one topdressing” technology in Damiao Village, Jiashan

In the high-yield demonstration field of Dahao Village, Jiashan, the "one base and one topdressing" fertilization technology of slow-release fertilizer has been successfully applied. The specific approach is: before machine transplanting, combine land preparation to apply a special compound fertilizer consisting of 40% slow-release nitrogen + 60% quick-acting nitrogen, 40 kg per mu (containing N-P₂O₅-K₂O is 25-12-15), providing about 70% of the nutrient requirements during the entire growth period. Ten days after transplanting, combine pest control and apply tillering-promoting fertilizer once, and apply 7.5 kg of special fertilizer containing 30% nitrogen, silicon and alginate per mu to promote effective tillering. After that, no conventional ear fertilizer will be added, and only a small amount of foliar fertilizer will be applied according to the seedlings before heading. Through this "one base and one topdressing" model, the total nitrogen application in the experimental field was about 15.4 kg/mu, which was more than 20% less than the conventional three-time fertilization of surrounding farmers, but the rice growth always maintained a balanced nutrient supply, and there was no lack of fertilizer in the middle and late stages. The demonstration results in 2022-2023 showed that the rice population treated with slow-release fertilizers one base and one topdressing grew neatly and robustly, with a moderate number of tillers at the base and an increased effective tillering rate. The number of panicles was comparable to that of the control, while the number of grains per panicle and the fruiting rate increased slightly, and the final yield was the same as that of conventional fertilization (Figure 3). At the same time, there was no excess nitrogen residue in the paddy field soil in the middle and late stages, the nitrogen content of rice panicles and stems and leaves was appropriate, the rice plants changed color normally, and there was no lodging or greed for green and late maturity (Wang et al., 2019). According to calculations, this technology reduces nitrogen fertilizer input by about 4 kg per mu compared with traditional fertilization, and the apparent nitrogen loss rate of paddy fields is significantly reduced (15% for conventional treatment, and reduced to less than 12% for one base and one topdressing treatment). The application in Dahao Village shows that the combination of slow-release fertilizer and mechanical deep side application can achieve the effect of one fertilization of rice equivalent to three fertilizations in the past, which not only simplifies the operation and saves labor costs, but also increases the net income by about 50 yuan per mu. At present, the "one base and one topdressing" fertilization technology has been promoted in more than 10 000 mu of Jiashan grain functional area, and has won unanimous praise from grain farmers. This proves that in areas with high-efficiency varieties and good mechanized planting foundations, the combination of advanced fertilizer products and simplified fertilization technology can effectively reduce fertilizer and increase efficiency.



Figure 3 The growth stages of rice

6.3 Field performance and evaluation data of efficient varieties

In the green high-yield demonstration in Jiashan, selected high-yield and high-efficiency varieties showed excellent field adaptability and yield-increasing effects. For example, the *indica-japonica* hybrid rice "Yongyou 33" was planted in 91.78 mu of the demonstration field, with an average yield of 778.5 kg per mu, an increase of about 5% over the control (the control is the average yield under local conventional management during the same period). The plants of "Yongyou 33" grow robustly, with large panicles and many grains, showing strong resistance to lodging and uniformity. Under the treatment of 20% nitrogen reduction, the variety still maintained excellent yield, only about 2% less than the conventional nitrogen treatment, showing a high nitrogen absorption and utilization efficiency (Zhou et al., 2020). The conventional late *japonica* rice variety "Jiahe 567" was planted in 14.83 mu of the demonstration field, with an average yield of about 625.4 kg per mu, and the rice quality was tested to reach the national standard high-quality level 3. Its nitrogen fertilizer partial productivity and agronomic efficiency are higher than the control variety Xiushui 134. "Jiahe 567" has good tillering and ear formation and high fruiting rate in medium fertility fields. It has a certain tolerance to insufficient nitrogen supply, and the yield has not decreased significantly under the condition of 15% nitrogen reduction (Sun et al., 2020). Another demonstration variety "Xiushui 1717" was planted in 7.51 acres, with an average yield of 598.3 kg per acre. The rice tastes soft and sticky and is popular in the market. This variety has moderate plant height and good color change at maturity. There is no greed for green and premature aging under one base and one topdressing. Comprehensive evaluation shows that varieties such as Yongyou 33 and Jiahe 567 have both high yield potential and high nitrogen efficiency, but each has its own advantages. Yongyou 33 is more suitable for high-input and high-yield cultivation, but is slightly inferior in stable yield and stress resistance; Jiahe 567 has a slightly lower yield but outstanding nitrogen efficiency, and is more suitable for medium fertility and green input mode. The demonstration also showed that there is a clear interaction between varieties and fertilization patterns: Yongyou 33 works best with side-deep slow-release fertilizer technology, which not only ensures nitrogen supply in the early and middle stages but also avoids excessive growth in the later stages; Jiahe 567 is more flexible to nitrogen reduction, and can achieve stable high yields with a 20%-30% nitrogen reduction when combined with moderately dense planting. Therefore, in large-scale promotion, the corresponding fertilization strategy should be selected according to the characteristics of the variety to achieve the best match between variety potential and cultivation measures, and achieve the win-win goal of high yield and high efficiency.

7 Concluding Remarks

Efficient nitrogen utilization in rice involves multiple processes from root absorption, assimilation and transport in the body to gene regulation. Recent studies have deepened our understanding of the core mechanism of this process: roots efficiently obtain soil nitrogen through ammonium/nitrate transporters, and rely on robust roots and rhizosphere nitrogen-fixing microorganisms to increase nitrogen supply; nitrogen ascends along the xylem and is

redistributed in the phloem in the plant, and later meets the filling demand through leaf-grain nitrogen transport. The coordination between source and sink determines nitrogen utilization efficiency and yield. Nitrogen metabolism and carbon metabolism are coupled to each other, and the C/N balance of the plant is maintained through feedback regulation at the transcriptional and metabolic levels, thereby ensuring normal growth and efficient nitrogen utilization. A number of key genes that control nitrogen efficiency have been identified: *OsNRT1.1B* and others determine the differences in nitrogen absorption among varieties, *OsGS1;1* and others affect nitrogen assimilation and reuse, and *OsNAC42*, *OsNLP4* and others are located upstream of the regulatory network, commanding the expression of nitrogen-responsive genes. These results have built a theoretical framework for efficient nitrogen utilization in rice. The core of rice NUE is to improve the productivity of unit nitrogen, which can be achieved by: enhancing the coordination between root absorption capacity and soil nitrogen supply, promoting efficient nitrogen transport between nutritional organs and reproductive organs, delaying the senescence of functional leaves to extend the assimilation period, and optimizing regulation to match nitrogen supply with crop growth needs. The above mechanism points out the direction for formulating nitrogen-saving and high-yield cultivation strategies and breeding improvements.

Promoting green and efficient fertilization technology is an important means to achieve sustainable agricultural development. On the one hand, these technologies can significantly reduce the use of chemical fertilizers, improve resource utilization efficiency, and reduce environmental load. For example, the application of slow-release fertilizers and soil-testing formula fertilization can reduce fertilizer input per unit area by 10%-30%, and nitrogen leaching and nitrous oxide emissions will be reduced accordingly, which will help mitigate agricultural non-point source pollution and greenhouse effect. On the other hand, green fertilization promotes healthy crop growth, soil fertility maintenance and biodiversity by improving the way crops are supplied with nutrients. For example, technologies such as returning straw to the field and replacing organic fertilizers can improve soil fertility while supplementing nutrients, achieving a virtuous cycle of carbon and nitrogen cycles in farmland. Another example is the combination of nitrogen reduction and plant protection technology, which reduces the use of pesticides and ensures the quality and safety of agricultural products and the stability of farmland ecosystems. Facts have proved that relying on scientific and technological innovation and reasonable policy guidance, my country has achieved the goal of zero growth in fertilizers from 2015 to 2020, and the input of fertilizers per unit of output in major grain crops has decreased year by year. This shows that the promotion of green fertilization technology has a significant effect on promoting the transformation of traditional agriculture to resource-saving and environmentally friendly agriculture. It can be foreseen that green fertilization will play a more critical role in the future sustainable development of agriculture: by matching with excellent rice varieties and cultivation patterns, it will support a modern rice farming system that takes into account high yield, high quality and ecological safety.

Looking to the future, the research and application of improving rice NUE should focus on the following aspects: First, deepen the study of molecular mechanisms, identify more nitrogen high-efficiency related genes and their action pathways, especially reveal the complex network of crop nitrogen metabolism regulation under different environmental conditions. Functional genomics and systems biology can be used to discover the role of currently unknown regulatory elements, such as non-coding RNA and epigenetic markers in nitrogen high efficiency, providing new ideas for molecular breeding. Second, accelerate the selection and breeding of nitrogen-efficient varieties and the creation of new germplasm. With the help of molecular marker-assisted selection and gene editing technology, excellent alleles are accurately introduced into the main varieties to achieve the directional improvement of low-nitrogen and high-yield traits. At the same time, focus on comprehensive trait cultivation, so that high-NUE varieties have both stress resistance and high quality characteristics to meet production needs. Third, integrate innovative cultivation technology to achieve the "three-in-one" optimization management of crops, soil and inputs. For example, develop intelligent fertilization systems, use sensor monitoring and model decision-making to dynamically supply nitrogen according to the actual needs of crops, and avoid shortages or surpluses. Another example is to improve water-fertilizer integration technology, reduce nitrogen losses while saving water, and improve nutrient utilization efficiency. Fourth, strengthen demonstration and promotion and policy support. In large-scale production, through the radiation and promotion of demonstration areas, let farmers

intuitively understand the effect of reducing nitrogen without reducing production, and enhance their willingness to adopt green fertilization technology. The government can continue to implement soil testing and formula fertilization subsidies, green agricultural projects, etc., to guide new agricultural operators to participate in technology promotion. Finally, a long-term mechanism for nitrogen fertilizer management should be established, including the formulation of regional and variety-specific nitrogen fertilizer recommendation standards and regulatory measures to ensure the continued achievement of the goal of reducing nitrogen fertilizer use and increasing efficiency. Efficient use of nitrogen is a key link in the "win-win" situation of increasing grain production and protecting the environment. With the development of biotechnology and digital agriculture, we have reason to believe that through a combination of research and promotion, the level of rice nitrogen management will continue to improve in the future, helping grain production move towards higher yields, higher efficiency and more sustainable goals.

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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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