

Progress of Research on Nitrogen Utilization in Rice

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ABSTRACT

Nitrogen (N) is one of the key factors that restrict rice (*Oryza sativa* L.) yield. The Green Revolution represented by the breeding of semi-dwarf crops greatly promoted agriculture grain yield. However, introduction of the semi-dwarf genes into rice resulted in the decreased nitrogen use efficiency (NUE). Lots of N fertilizer were input to maintain high rice yield and thus generated tremendous pollution problems. As we known, N use efficiency is a typical quantitative trait. It is not easy to identify and accurately excavate the genes which control nitrogen use efficiency by the traditional genetics' methods. This review highlight the recent advances in nitrogen use efficiency of rice, especially the researches in molecular genetics and genomics related field, which reveal the genetic control of nitro-gen use efficiency of rice and lay the foundation for rice nitrogen efficient molecular marker assisted breeding. Furthermore, mining elite genes within rice is of importance for uncovering genetic mechanism of nitrogen use efficiency in rice Which is a new strategy for sustainably increasing grain yield, yet reducing environmentally degrading agricultural N use. In this paper, the progress of genome strategies for mining genes related to nitrogen absorption efficiency is highlight stated.

Keywords: *Oryza Sativa*; Genome Study; Plant Type; Nitrogen Fertilizer

Introduction

Rice (*Oryza sativa* L.) is one of the largest and most important food crops grown in China. N fertilizer plays the most significant role in rice growth development and high yield formation, which accounting for more than 60% of all types of chemical fertilizer inputs. So increasing nitrogen fertilizer inputs is one of the most effective measures to improve rice yields. Since the 1980s, 35% of the world's nitrogen fertilizer has been consumed on 7% of the world's arable land. A study showed that China's annual grain production increased by 86% from 1981 to 2015, [1-5] while nitrogen fertilizer consumption increased by nearly two times. China's current average nitrogen fertilizer application for rice is 75% higher than the world, and the absorption rate of nitrogen fertilizer is only 33%, resulting in a great waste of energy and in-creasing food costs. How to reduce nitrogen fertilizer inputs in agricultural production meanwhile continue to improve crop yields? It has become a major issue for the sustainable development of China's agriculture.

Nitrogen Fertilizer Utilization

Nitrogen fertilizer is an essential nutrient for plant growth and development which play an irreplaceable role in stable and high yield of crops. While the yield formation is closely related to the relevant agronomic traits in crop growth and development. In the 1960s, the "Green Revolution", characterized by dwarf breeding, substantially increased rice yields by increasing nitrogen fertilization, which solved the problems of plant collapse and yield reduction. [5-10] How-ever, on the other hand, rice became more dependent on chemical fertilizers and became less efficiency in using nitrogen fertilizers. More than 40 years later plant molecular biology and genomics revealed that mutations in the *sd1* gene, a semi-dwarf trait, reduced plant height by suppressing plant growth and weakened plant response to nitrogen. Lots of research has been conducted by scholars on how to improve the efficiency of nitrogen fertilizer use in crops. Tirol Padre A (1996) concluded that rice can be defined as nitrogen efficient genotypes if the yield of rice is higher than the average yield of rice in the same reproductive period regardless of the nitrogen supply capacity.

Yali Zhang (2008) considered that nitrogen-efficient varieties could achieve higher yields at both low and high nitrogen levels. But whether the effect of nitrogen directly leads to high rice yield is not known. It is not completely clear about the relationship between nitrogen uptake or utilization and physiological activity, trait in different varieties of rice. The molecular genetic mechanisms are not fully understood (Jauhar Ali et-al,2018).

Relationship Between Nitrogen Fertilization and Rice Traits

The research on ideal plant type was systematically proposed by Donald in Australia in 1968. In the field of rice breeding, several breeders have proposed the ideal plant type for high yield of rice, and all of them invariably mentioned the selection and breeding of rice morphology. Nitrogen, as the most important structural material, is involved in the synthesis of cystoid membrane proteins, chlorophyll synthesis and Rubisco enzyme synthesis in chloroplasts during photosynthesis (Wang, Yuxing, 2018). Many previous studies have focused on the changes in chlorophyll content and leaf color caused by nitrogen fertilization, leading to the perception that leaf length and leaf width affect the area of photosynthesis, achlorophyll content affects the efficiency of photosynthesis (Shuangbing Zhu, 2020), which is significantly correlated with net photosynthetic rate. With the development of molecular biology techniques, more and more genes related to plant morphogenesis have been identified. Some studies have shown that indica rice sword leaf length and width are controlled by at least two pairs of genes. But japonica rice sword leaf may be controlled by micro-effective polygenes (Madapeng 1989; Yan, Yueming 1990). Leaf type-related traits in rice are typically quantitative traits, N use efficiency is also a complex multi-gene controlled quantitative trait which is susceptible to growth environment.

Current Status of Research on Nitrogen Fertilizer Utilization in Rice

Research on rice is closely related to the development of biotechnology theory. The problem of nitrogen fertilizer utilization efficiency arose in the 1960s, when the "Green Revolution", characterized by dwarf breeding, solved the problems of plant collapse and yield reduction caused by heavy fertilizer application and substantially increased rice yield. [10-15] However, it was accompanied by the disadvantages of reduced nitrogen fertilizer utilization efficiency and high dependence on chemical fertilizers. In the 1980s, the development of biotechnology gave rise to molecular marker-assisted breeding, which made traditional genetic breeding somewhat traceable. It was recognized that the mutation of Semi-dwarf1 (SD1), a "green revolution" gene in rice, reduced plant height by inhibiting plant growth and weakened plant response to nitrogen. In 1998, the "International Rice Genome Sequencing Project" aimed at research on important agro-

nomical traits such as high yield, high quality and resistance to stress, which reserve excellent genetic resources for the use of nitrogen fertilizer in rice. Since 2000, most domestic and international studies have focused on the identification of nitrogen efficiency quality of rice and QTL localization of traits related to low nitrogen tolerance in rice. However, seldom studies from the genome level (Rao IS, et al. [1]). How to break through the bottleneck of traditional genetic breeding technology. How to apply rice molecular genetics and functional genomics to low nitrogen breeding improvement. The accurate rice molecular design breeding has become a major problem in the new era. At present, some progress has been made in the localization and cloning of genes related to nitrogen use efficiency in rice. To summarize, the nitrogen-related genes that have been cloned can be broadly classified into four types.

The first category of genes is characterized by genes encoding nitrate transporter proteins, such as the nitrate transporter OsNRT2.3b gene. High expression of such genes in rice enhances nitrogen uptake. The nitrogen efficient utilization gene NRT1.1B encoding a nitrate transporter protein was cloned from indica rice in 2015, which differed by only one amino acid between indica and japonica rice. Deletion and natural variation of this gene were found to significantly affect the microorganisms associated with the nitrogen cycle in the rice root system in 2022. An allele of the NAC-42-activated nitrate transporter protein gene OsNPF6.1 was identified in 2019, which could improve rice N use efficiency and yield. This method reduces N fertilizer use, provides a genetic resource and a new strategy for efficient N use and high-yield breeding in rice. The second category of genes is characterized by genes that utilize ammonium nitrogen sources. In 2018, OsNRT1.1A, a key gene for controlling nitrogen efficiency, high yield and early maturity in rice, was discovered. Overexpression of this gene induced by ammonium can significantly shorten rice maturation time, increase rice biomass and yield and solve the problem of "greedy green and late maturity". The third category of nitrogen metabolism enzymes and hormone-related genes. In 2020, researchers cloned NGR5, a key gene that controls the efficient use of nitrogen fertilizer in rice, which is a positive regulator of plant response to nitrogen and can increase the number of tillers in rice. This gene can increase the number of tillers in rice without changing the semi-dwarf trait of "Green Revolution" and promote the uptake and utilization of nitrogen fertilizer, which can further improve the efficiency of nitrogen fertilizer utilization while reducing nitrogen fertilizer input. The fourth category is related to the physiological mechanism of N uptake and translocation utilization. The higher level expression of the growth regulator GRF4 in sd1 varieties could improve the efficiency of N utilization while maintaining the characteristics of resistance to collapse and high yield conferred by the semi-dwarf trait, which providing a technical path-way for the new green revolution. 2019 study found that nitrate was negatively regulated by its receptor protein

NRT1.1B through the regulation of the phosphorus signaling pathway. SPX4, a key negative regulator of the phosphorus signaling pathway, activates both NLP3 and PHR2-dependent nitrogen signaling pathways to promote synergistic uptake and utilization of nitrogen in rice. In summary, the genome of rice is large and complex, so the key response genes need to be further explored, include genes related to nitrogen uptake, transport and assimilation. Their expression have been studied in rice from a molecular perspective and further mining the related genes is needed. These studies were obtained based on biparental and mutant cloning, still need in-depth systematic research on the mining of nitrogen efficient and beneficial alleles.

The Latest Developments of Domestic and International

Nitrogen uptake and utilization in rice is a complex trait controlled by multiple genes, while the plant response to low nitrogen signals is also multilevel. Most QTL studies on nitrogen uptake are limited by low genetic diversity, low marker density and small population size. In recent years, genome-wide association studies (GWAS) have been used to dissect the genetic basis of complex traits and have been successfully used to identify genomic regions affecting numerous traits in many crop species. [15-17] Scientists selected 517 copies of two major subspecies cultivated rice and performed GWAS analysis on 14 agronomic traits in indica rice varieties. The candidate loci for some related genes were identified. These findings provide important basic data for rice genetics research and rice breeding using natural populations. In 2017, based on the functional genes of key agronomic traits of rice, developed efficient breeding strategies through molecular design breeding, which greatly improved breeding efficiency, shortened breeding time, and rapidly aggregated excellent traits, and the new rice varieties bred with this system have gradually made a significant impact on rice production in China.

In 2018, A key gene ARE1 that controls nitrogen use efficiency in rice was identified. This gene encodes a chloroplast-localized protein, and its functional deletion in ARE1 mutants under nitrogen-limited conditions enhances nitrogen use efficiency, delays rice senescence, and improves rice yield. The loss of function in ARE1 mutant enhanced N use efficiency, delayed rice senescence and improved rice yield under N limitation. The mutant provides an important genetic resource for breeding efficient nitrogen-using rice. In 2019, OsNR2, a gene that can improve rice yield and nitrogen use efficiency mechanism was discovered. In 2020, the molecular mechanism of the transcription factor MYB61 to promote nitrogen utilization and improve rice yield. The study reveals a direct molecular linkage between carbon and nitrogen metabolism. Identifying key nodes that integrate carbon and nitrogen metabolism can improve the efficiency of nitrogen used in rice and other crops, which can achieve "reduce and increase efficiency" in agricultural production. It is molecular tools and new ways to improve N use efficiency in rice and other crops, which can achieve "reduction and efficiency" in agricultural production.

Application of Genomics in Rice Molecular Breeding

Genome-wide association analysis is an important method for unknown gene discovery research. It may be more comprehensive and reliable for scanning multiple traits and the entire genome. The continuous development of sequencing technology and the decreasing cost of sequencing have played a great role in promoting the whole-genome association analysis. Currently, genome-wide association analysis is a popular research method. Its advantages are short cycle time, wide range and high precision, which focus on the cloning of more genes affecting nitrogen efficiency using linkage analysis to provide genetic resources and lay the foundation for molecular genetic improvement of rice.

1. The selection methods for complex quantitative traits in the last decade have gone from phenotypic selection to molecular marker-assisted selection for a few master-effect loci. These methods have not really been successfully implemented into breeding practices for complex traits.
2. In previous studies, the genetic segregation of two parental populations was often unable to compare the allelic diversity of QTLs and determine the best favorable alleles for molecular breeding, which was not conducive to further fine localization of QTLs and gene cloning and difficult to guide the genetic improvement of breeding populations.
3. Association analysis can save time (no need to construct a mapping population, as association analysis can be performed using natural population), breadth (multiple traits can be examined simultaneously for association analysis), and accuracy compared with traditional chain mapping.

In conclusion, better understand the molecular mechanism of nitrogen utilization in rice, provide new reference loci for future plant type design breeding. Which also provide an important basis for cloning of new candidate genes and make some contribution to further improve and utilize excellent rice variety resources.

Declarations

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