Analysis of Rice Nitrogen Use Efficiency and Postprint Utilization Based on Recombinant Inbred Lines Population

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Abstract

This study employed a rice recombinant inbred line (RIL) population as experimental material to conduct a field experiment with two nitrogen treatments: no nitrogen application and low nitrogen application (150 kg·hm 2), investigating the distribution characteristics of nitrogen uptake, utilization, and major agronomic traits in the rice RIL population, and elucidating inter-trait relationships through correlation, cluster, and principal component analyses to provide a theoretical basis for breeding new rice varieties with high nitrogen use efficiency. The results indicated that nitrogen use efficiency traits in the rice RIL population exhibited relatively large coefficients of variation under the 150 kg·hm 2 nitrogen condition; nitrogen application enhanced nitrogen content in panicles, stems, and leaves, and increased total dry matter per plant (including panicle weight, stem weight, and leaf weight per plant). Under both nitrogen environments, nitrogen dry matter production efficiency was positively correlated with plant height, panicle length, stem weight per plant, and total dry matter per plant, but negatively correlated with nitrogen content in stems, leaves, and panicles; nitrogen grain production efficiency was positively correlated with grain weight per plant, seed setting rate, 1000-grain weight, total spikelets per panicle, and panicle length, but negatively correlated with stem weight per plant, leaf nitrogen content, leaf weight per plant, and total nitrogen accumulation per plant. Stepwise regression analysis revealed that stem nitrogen content, panicle nitrogen content, and stem weight per plant significantly affected nitrogen dry matter production efficiency, whereas panicle number, total spikelets per panicle, and seed setting rate more significantly influenced nitrogen grain production efficiency. Principal component analysis demonstrated that higher nitrogen use efficiency was associated with lower plant nitrogen content, particularly in stems. Therefore, under low nitrogen field conditions, screening should focus on heavy-panicle type lines (characterized by longer panicles, more total spikelets, and higher seed setting rates) with greater plant height and stem weight, combined

with lower nitrogen content in stems and panicles, especially lower stem nitrogen content, to facilitate breeding of nitrogen-efficient rice varieties. Thirteen superior lines selected from this population, including nitrogen-efficient line Q149 and nitrogen-inefficient line Q114, can serve as excellent genetic resources for future research.

Full Text

Preamble

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Analysis of Rice Nitrogen Use Efficiency Based on Recombinant Inbred Line Population

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Abstract: Nitrogen is the most critical input limiting rice productivity. Due to increasing fertilizer costs and environmental concerns, nitrogen use efficiency (NUE) is hotly debated in the scientific community. To explore the absorption, utilization, and distribution patterns of nitrogen in recombinant inbred lines (RILs), a field experiment was conducted to evaluate the potential NUE of rice (*Oryza sativa* L.). The split-plot experiment had two treatments (one without nitrogen fertilizer and the other with 150 $kg \cdot hm^{-2}$ of nitrogen) for populations of RILs, the parents, and the check line Q149. The relationship between NUE and the main agronomic characteristics of the RIL population was determined using statistical correlation, clustering, and principal component analyses. The results showed that the variation coefficient of NUE was larger under 150 $kg \cdot hm^{-2}$ nitrogen treatment than under non-nitrogen treatment. Proper increase in nitrogen fertilization was beneficial to nitrogen content in rice panicle, stem, and leaf, and to total dry matter weight of single plant. Significantly positive correlations were noted between dry matter production efficiency and plant height, panicle length, stem weight per plant, and total dry matter weight of single plant under both nitrogen treatments. Also under both treatments, significantly negative correlations were noted between dry matter production efficiency and the contents of nitrogen in rice stem, leaf, and panicle. There were positive correlations between grain production efficiency and grain weight per plant, seed setting rate, thousand-seed weight, total number of grains per panicle, and spike length. Equally, there were negative correlations between grain production efficiency and stem weight per plant, leaf nitrogen content, leaf weight per plant, and total amount of nitrogen per plant. Stepwise regression analysis indicated that nitrogen content in stem and panicle, and stem weight per plant had significant effects on dry matter NUE. However, the effects of number of panicles per plant, number of grains per panicle, and seed setting rate on grain NUE were more significant in the two treatments. Principal component analysis showed that the nitrogen content in rice plant, especially in stem, decreased with increasing NUE. Therefore, in low-nitrogen field conditions, it was necessary to select heavy-panicle type varieties that were high in stem weight (including long panicle length, more grains per panicle, and high seed-setting rate) in breeding programs. Furthermore, lower nitrogen content in stem and panicle (especially in stem) benefited the breeding for high NUE. Based on the study, 7 rice lines with high NUE (e.g., Q149) and 6 lines with low NUE (e.g., Q114) were selected for special germplasm in rice breeding projects.

Keywords: Rice; Recombinant Inbred Line; Nitrogen fertilization rate; Nitrogen use efficiency; Agronomic trait

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Nitrogen is one of the key factors limiting rice yield, while excessive nitrogen fertilizer input has caused significant harm to the ecological environment and sustainable agricultural development. Breeding new rice varieties with high nitrogen use efficiency has become an important objective for breeders. In the past, genetic selection was often conducted under high nitrogen background to cope with high nitrogen input, but high nitrogen masks genotypic differences in nitrogen absorption and utilization. Therefore, selection under low nitrogen conditions is considered an important approach for breeding rice varieties with high nitrogen use efficiency. Previous studies have shown that under no nitrogen application, the correlations between grain weight per plant and plant height, panicle number, grains per panicle, and straw weight per plant, as well as the nitrogen response index of grain weight per plant with panicle number, grain weight per plant, straw weight per plant, and grain-straw ratio, are more closely correlated than under low or normal nitrogen levels. Rice plant nitrogen accumulation increases rapidly with increasing nitrogen application rates, but nitrogen absorption remains basically unchanged when nitrogen application exceeds 225 $kg \cdot hm^{-2}$. Most previous research has focused on the relationship between nitrogen use efficiency and yield and important agronomic traits. For example, high-yielding rice genotypes have higher nitrogen accumulation, nitrogen absorption rate before heading, and nitrogen use efficiency at various growth stages. Rice nitrogen use efficiency is significantly negatively correlated with nitrogen content in stems and leaves at maturity. However, most studied materials were collected from popularized varieties or backbone parental materials with different genetic backgrounds and mostly excellent agronomic traits, including hybrid varieties with heterosis. For breeding new nitrogen-efficient rice varieties, segregating populations include both superior and inferior groups. Therefore, this study utilized an F_9 recombinant inbred line (RIL) population derived from a cross between a nitrogen-efficient rice parent and a nitrogen-inefficient rice parent to investigate nitrogen absorption and utilization parameters and main agronomic traits. Correlation and principal component analyses were conducted between these parameters and nitrogen use efficiency to clarify the trait characteristics of nitrogen-efficient rice populations and identify highly contributive trait parameters for evaluating nitrogen use efficiency. This provides a theoretical basis for current breeding programs that still employ traditional methods for selecting nitrogen-efficient new varieties.

Materials and Methods

Experimental Materials and Site

The field experiment was conducted from May to October 2010 at the Feidong Yangdian Experimental Base in Anhui Province. The tested soil was yellow-white soil with basic physicochemical properties as follows: total nitrogen 0.95 $g \cdot kg^{-1}$, alkaline hydrolyzable nitrogen 116 $mg \cdot kg^{-1}$, available phosphorus 15.1 $mg \cdot kg^{-1}$, available potassium 135 $mg \cdot kg^{-1}$, and organic matter content 15.33 $g \cdot kg^{-1}$. The experimental material consisted of a recombinant inbred line (RIL) population (Dasanbyeo/TR22183; F_9, provided by Shanghai Academy of Agricultural Sciences) derived from a cross between the nitrogen-efficient *indica* rice variety 'Dasanbyeo' and the temperate *japonica* nitrogen-inefficient rice variety 'TR22183' . The population comprised 163 lines, and statistical analysis was performed on 138 lines after removing outliers, along with parental materials and the newly bred control line Q149.

Experimental Design and Management

A two-factor split-plot design was employed with nitrogen treatments as main plots and lines as subplots. The main plots consisted of two nitrogen application rates: no nitrogen (N0) and low nitrogen [N150, 150 $kg(N) \cdot hm^{-2}$]. Main plots were separated by plastic film barriers. The split plots were arranged in a randomized complete block design with three replications. Each material was planted in 5 rows with 11 plants per row at a spacing of 16.7 cm × 20 cm, with single seedling transplanting. Urea was used as the nitrogen source and applied in three splits at a ratio of base fertilizer:tillering fertilizer:panicle fertilizer = 4:3:3. Phosphorus fertilizer (calcium magnesium phosphate containing 15% P_2O_5) was applied at 375 $kg \cdot hm^{-2}$, and potassium fertilizer (potassium chloride containing 60% K_2O) at 225 $kg \cdot hm^{-2}$, both as base fertilizers. Seeds were sown on May 7 and transplanted on June 6. Pest, disease, and weed control, as well as irrigation, were managed according to standard field production practices.

Sampling and Measurements

At maturity, panicle number (tiller number) was surveyed for each plot. Five representative plants were sampled from each subplot. After removing roots, stems, leaves, and panicles were separated, dried in an oven at 105 °C, and their dry weights measured. The samples were then ground and analyzed for nitrogen content in each organ using an automatic nitrogen analyzer (BUCHI 339). Another five plants were sampled for yield component analysis, including plant height, panicle number, panicle length, total grains per panicle, filled grains per panicle, thousand-grain weight, and grain weight per plant.

Nitrogen dry matter production efficiency (NDMPE) was calculated as the ratio of total plant dry matter weight per unit area to total plant nitrogen accumulation per unit area. Nitrogen grain production efficiency (NGPE) was calculated as the ratio of grain yield per unit area to total plant nitrogen accumulation per unit area.

Data Analysis

SPSS 19.0 statistical software was used for descriptive statistics, correlation analysis, cluster analysis, and principal component analysis of the RIL population traits.

Results

Trait Variation in the RIL Population

Variation coefficients of traits provide reference for target trait selection in variety improvement and implementation of cultivation measures. Statistical analysis of trait variation among 138 lines of the Dasanbyeo/TR22183 RIL population under different nitrogen levels showed that nitrogen dry matter production efficiency and nitrogen grain production efficiency exhibited large differences under both nitrogen treatments, with larger variation coefficients under the 150 $kg \cdot hm^{-2}$ condition. Nitrogen dry matter production efficiency ranged from 51.15 to 83.02 $g \cdot g^{-1}$, while nitrogen grain production efficiency ranged from 4.24 to 46.52 $g \cdot g^{-1}$. Nitrogen application promoted the increase of average nitrogen content in the population and improved main agronomic traits, except for thousand-grain weight which decreased. Differences in trait means between the two nitrogen levels were significant or highly significant for all traits except panicle number, panicle length, and total grains per panicle .

Performance of High and Low NUE Lines

Using K-means clustering with nitrogen use efficiency traits (nitrogen dry matter production efficiency and nitrogen grain production efficiency) as variables, the population lines were clustered and group characteristics were analyzed. After removing outliers, the 138 lines were divided into three groups . Under N150 conditions, Group 1 contained 65 lines, Group 2 had 37 lines, and Group 3 comprised 36 lines (including the newly bred line Q149). ANOVA and multiple comparison analysis of the clustering results showed no significant differences within groups but significant differences between groups, with many traits reaching significant levels. Group 3 lines exhibited significantly higher nitrogen use efficiency and grain weight per plant than the other two groups, indicating that high-yielding, nitrogen-efficient lines could be selected from the nitrogen-efficient group. Total nitrogen accumulation per plant showed no significant differences between groups. The process of constructing recombinant inbred lines is also a process of self-selection for new varieties. Based on clustering results under both nitrogen conditions, seven lines (including the newly bred Q149, plus Q096, Q153, Q048, Q091, Q128, and Q22) consistently appeared in the nitrogen-efficient group under both nitrogen levels and were preliminarily identified as nitrogen-efficient lines. Six lines (Q114, Q104, Q060, Q118, Q135, and Q151) consistently appeared in the nitrogen-inefficient group and were designated as nitrogen-inefficient lines . The main traits of these lines along with the two parents and control line Q149 are presented in Table 3. Under nitrogen-efficient conditions, the seven nitrogen-efficient lines showed average nitrogen dry matter production efficiency and nitrogen grain production efficiency that were 36.12% and 33.81% higher, respectively, than the six nitrogen-inefficient lines. The newly bred line Q149 exhibited 6.22% and 2.57% higher values than its nitrogen-efficient parent Dasanbyeo, showing certain transgressive heterosis. Comparison between the two groups also revealed that nitrogen-efficient rice under low nitrogen conditions had higher plant height, grain weight per plant, and biomass, larger panicles, and lower nitrogen content in stems and leaves, particularly significantly reduced stem nitrogen content.

Relationships Between NUE and Other Traits

Analysis of relationships between nitrogen use efficiency and main agronomic traits showed that under both N0 and N150 conditions, nitrogen dry matter production efficiency was positively correlated with plant height (r=0.593**, r=0.271**), panicle length (r=0.318**, r=0.186*****), stem weight per plant (r=0.485, r=0.320), and total dry matter weight per plant (r=0.426, r=0.331), and negatively correlated with stem nitrogen content (r=−0.823, r=−0.813), leaf nitrogen content (r=−0.196*****, r=−0.453**), and panicle nitrogen content (r=−0.469**, r=−0.708**). Nitrogen grain production efficiency was positively correlated with grain weight per plant (r=0.842**, r=0.613**), seed setting rate (r=0.773**, r=0.353**), thousand-grain weight (r=0.317**, r=0.264**), total grains per panicle (r=0.191*, r=0.326**), and panicle length (r=0.20*, r=0.304**), and negatively correlated with stem weight per plant (r=−0.530**, r=−0.351**), leaf nitrogen content (r=−0.313**, r=−0.382**), leaf weight per plant (r=−0.299**, r=−0.359**), and total nitrogen accumulation per plant (r=−0.180*, r=−0.366**) . Stepwise regression analysis revealed that under both nitrogen levels, stem nitrogen content, panicle nitrogen content, and stem weight per plant entered the regression equation for nitrogen dry matter production efficiency, while panicle number, total grains per panicle, and seed setting rate entered the regression equation for nitrogen grain production efficiency with larger standardized coefficients, indicating their greater importance.

Principal Component Analysis

Principal component analysis is a technique for studying the common relationships among correlated variables; variables with high scores on principal components have greater influence. Based on original data of nitrogen absorption and utilization traits and other main agronomic traits, eigenvectors and contribution rates of each principal component were calculated using SPSS 19.0 software, with components having eigenvalues greater than 1 extracted . Variables were assigned to different principal components based on the location of their maximum absolute value across factors. The cumulative contribution rate of five components reached 78.60%, reflecting most information from the original variables.

The first principal component had the largest eigenvector value for stem nitrogen content, followed by panicle nitrogen content and leaf nitrogen content, while nitrogen dry matter production efficiency and nitrogen grain production efficiency had large negative eigenvector values. This component reflected nitrogen utilization efficiency and was termed the nitrogen utilization factor, indicating that high nitrogen utilization efficiency was associated with low plant nitrogen content, especially in stems. The second principal component had the largest eigenvector for total dry matter weight per plant, followed by leaf weight per plant, stem weight per plant, and total nitrogen accumulation per plant, all related to nitrogen absorption, while nitrogen grain production efficiency had a high negative value. Thus, the second component was termed the nitrogen absorption factor. The third component had the largest eigenvector for grain weight per plant, followed by seed setting rate and panicle weight per plant, with stem weight per plant having a high negative value, and was termed the single plant yield factor. The fourth and fifth components were mainly contributed by plant height, panicle number, panicle length, and total grains per panicle, and could be termed yield components factors.

Discussion and Conclusion

Under high nitrogen background, nitrogen accumulates excessively in non-yield organs such as stems and leaves, causing "luxury nitrogen consumption" in rice. Appropriately reducing nitrogen fertilizer application not only does not reduce yield but may even increase it. Under field conditions without nitrogen or with low nitrogen, rice can fully express the genetic potential for nitrogen efficiency differences, but excessive selection pressure results in small variation coefficients of nitrogen use efficiency among different genotypes, preventing the advantages of efficient genotypes from being expressed and hindering screening for nitrogen-efficient varieties. This study demonstrated that appropriate nitrogen application promoted increased nitrogen content in panicles, stems, and leaves, and improved total dry matter weight per plant (including panicle weight, stem weight, and leaf weight per plant). Nitrogen use efficiency traits showed larger variation coefficients under low nitrogen (150 $kg \cdot hm^{-2}$) conditions, indicating that screening for nitrogen efficiency genotypic differences in

RIL populations follows the same field screening patterns as other materials under low nitrogen conditions.

Previous studies have found that under 180 $kg \cdot hm^{-2}$ nitrogen treatment, there were generally no significant differences between high-efficiency and low-efficiency varieties in aboveground nitrogen accumulation at different growth stages. This study showed that total nitrogen accumulation per plant was not significantly correlated with nitrogen dry matter production efficiency under both nitrogen levels, and group differences were not significant in cluster analysis, similar to results reported by Yan et al. but different from Cao et al., possibly due to differences in material selection. Through clustering, 13 typical nitrogen-efficient lines (such as Q149) and nitrogen-inefficient lines (such as Q114) were selected from the population, providing a material basis for further research on physiological, biochemical, and molecular biological mechanisms of nitrogen efficiency in rice.

A rapid and reliable method for identifying nitrogen-efficient rice germplasm is essential for breeding nitrogen-efficient rice varieties. Numerous studies have reported indicators for identifying nitrogen-efficient rice. Plant height is generally considered an indicator for predicting nitrogen use efficiency (nitrogen dry matter production efficiency) in rice varieties, while plant height, effective panicle number, biological yield, single plant yield, and seed setting rate can serve as screening indices for low-nitrogen tolerant rice materials. This study showed that plant height significantly affected nitrogen dry matter production efficiency but had little effect on nitrogen grain production efficiency, which was positively correlated with grain weight per plant. Principal component analysis indicated that the first principal component was the nitrogen utilization factor. High nitrogen use efficiency was associated with low plant nitrogen content, especially stem nitrogen content. The second principal component was the nitrogen absorption factor, with total nitrogen accumulation per plant closely related to panicle weight, stem weight, and leaf weight per plant. Previous research suggests that yield serves as an indirect selection trait for nitrogen use efficiency in nitrogen-efficient rice breeding, with good selection effects under both nitrogen-applied and nitrogen-free conditions. While considering total nitrogen absorption and grain yield, agronomic trait selection should also focus on large panicles, high harvest index, and high seed setting rate.

Integrating correlation and principal component analyses, this study concluded that under low nitrogen conditions, selecting lines with higher plant height, longer panicles, heavier stem weight, and lower nitrogen content in stems and panicles would improve nitrogen dry matter production efficiency. Selecting lines with more panicles, higher total grains per panicle and seed setting rate, and lower leaf nitrogen content and leaf weight per plant would improve nitrogen grain production efficiency. Therefore, in breeding nitrogen-efficient rice varieties under field low nitrogen (150 $kg \cdot hm^{-2}$) conditions, emphasis should be placed on screening heavy-panicle type lines with higher plant height and heavier stem weight (longer panicles, more total grains per panicle, and higher seed setting rate), combined with lower nitrogen content in stems and panicles, especially lower stem nitrogen content, which will facilitate the breeding of new nitrogen-efficient rice varieties.

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Note: Figure translations are in progress. See original paper for figures.