Genotypic Differences in Nitrogen Nutrition Characteristics of Rice Seedlings and Their Comprehensive Evaluation Postprint

Authors: Chen Chen, Gong Haiqing, Zhang Jingzhi, Xu Yujun, Gao Hongjian

Date: 2017-11-07T00:00:00+00:00

Abstract

Excessive application of nitrogen fertilizer not only causes substantial nitrogen loss, but also increases agricultural production costs, posing a tremendous threat to the ecological environment. Screening nitrogen-efficient genotype rice varieties is an effective approach to improve nitrogen use efficiency and reduce environmental pollution. In this study, a nutrient solution culture method was employed to investigate the differences in nitrogen absorption and accumulation at the seedling stage among 55 rice varieties (lines) under the same nitrogen supply level (40 mg·L 1) but different nitrogen forms (NH4+-N and NO3 --N). The membership function method was adopted to standardize the evaluation indices, and based on the comprehensive nitrogen efficiency value, hierarchical clustering heatmap analysis was performed to classify the nitrogen efficiency types of the 55 rice varieties, providing a basis for screening nitrogen-efficient rice varieties. Under NH4+-N and NO3 --N cultivation, significant differences were observed among different rice varieties in whole-plant biomass, shoot biomass, root biomass, root nitrogen content, and shoot nitrogen accumulation, with coefficients of variation ranging from 0.69–0.80 and 0.57–0.74, respectively. Factor analysis revealed that the principal component patterns were identical under NH4+-N and NO3 --N cultivation conditions: the first principal component was determined by whole-plant biomass, shoot biomass, root biomass, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation, primarily reflecting plant biomass and nitrogen accumulation indices; the second principal component was determined by nitrogen content in different organs. Based on the comprehensive analysis of variation characteristics in nitrogen absorption and accumulation at the rice seedling stage and factor analysis, whole-plant biomass, shoot biomass, root biomass, and shoot nitrogen accumulation were selected as comprehensive evaluation indices for nitrogen efficiency at the rice seedling stage. According to the

comprehensive nitrogen efficiency values calculated by the membership function method and the hierarchical clustering heatmap fitted using squared Euclidean distance, the 55 tested rice varieties could be divided into three categories: nitrogen-efficient, nitrogen-moderately-efficient, and nitrogen-inefficient types, accounting for 10.91%, 27.27%, and 61.82% of the total tested varieties, respectively. Under NH4+-N and NO3 --N supply conditions, 'Guangliangyou 3905', 'Yongyou 9', 'Zhongxian 2503', 'II You 602', 'Liangyou 766', and 'Shenliangyou 1813' were preliminarily identified as nitrogen-efficient varieties.

Full Text

Evaluation of Nitrogen Nutrition Characteristics of Different Rice Cultivars at Seedling Stage

CHEN Chen, GONG Haiqing, ZHANG Jingzhi, XU Yujun, GAO Hongjian
School of Resources and Environment, Anhui Agricultural University, Hefei 230036, China

Abstract

Excessive application of nitrogen (N) fertilizers leads to nitrogen loss, increases agricultural production costs, and poses significant threats to the ecological environment. Screening rice cultivars with high N efficiency represents an effective approach for improving nitrogen use efficiency and reducing environmental pollution. This study investigated differences in nitrogen absorption and accumulation among 55 rice cultivars (lines) at the seedling stage using hydroponic culture at a uniform nitrogen supply level of 40 mg · L 1 with different nitrogen forms (NH -N and NO -N). Evaluation indices were standardized using the subordinate function method, and the 55 rice cultivars were classified based on comprehensive nitrogen efficiency values and hierarchical cluster heatmap analysis, providing a foundation for screening nitrogen-efficient rice varieties.

Under NH -N and NO -N culture conditions, significant differences were observed among rice cultivars in whole-plant biomass, shoot biomass, root biomass, root nitrogen content, and shoot nitrogen accumulation, with coefficients of variation ranging from 0.69 to 0.80 and 0.57 to 0.74, respectively. Factor analysis revealed that under both NH -N and NO -N conditions, the first principal component was determined by whole-plant biomass, shoot biomass, root biomass, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation, primarily reflecting plant biomass and nitrogen accumulation indicators. The second principal component was determined by nitrogen content in different organs. Based on the variation characteristics of nitrogen absorption and accumulation at the seedling stage and factor analysis, whole-plant biomass, shoot biomass, root biomass, and shoot nitrogen accumulation were selected as comprehensive evaluation indices for nitrogen efficiency at the seedling stage.

According to the comprehensive nitrogen efficiency values calculated by the subordinate function method and hierarchical cluster heatmap analysis using squared Euclidean distance, the 55 tested rice cultivars were divided into three categories: nitrogen-efficient, nitrogen-moderately efficient, and nitrogen-inefficient types, accounting for 10.91%, 27.27%, and 61.82% of the total cultivars, respectively. Under NH -N and NO -N supply conditions, 'Guangliangyou 3905' , 'Yongyou 9' , 'Zhongxian 2503' , ' you 602' , 'Liangyou 766' , and 'Shenliangyou 1813' were preliminarily identified as nitrogen-efficient cultivars.

Keywords: rice cultivars; seedling stage; nitrogen form; nitrogen efficiency; cluster analysis

---

1.2 Measurement Items

When rice seedlings reached the six-leaf stage, shoot and root samples were harvested separately. Portions of shoot and root samples were killed at 105 °C for 30 minutes, then dried to constant weight at 75 °C to determine shoot biomass (SB) and root biomass (RB). Whole-plant biomass (WPB), whole-plant nitrogen content (WPNC), and whole-plant nitrogen accumulation (WPNA) were calculated accordingly. Rice shoot and root samples were digested using H SO -H O , and shoot nitrogen content (SNC) and root nitrogen content (RNC) were determined by the Kjeldahl method. Shoot nitrogen accumulation (SNA), root nitrogen accumulation (RNA), and whole-plant nitrogen accumulation were calculated as the product of dry weight and nitrogen content for shoots, roots, and whole plants, respectively.

1.3 Calculation Method for Comprehensive Nitrogen Efficiency Value

The subordinate function method was employed for comprehensive analysis of nitrogen efficiency values. This method standardizes rice nitrogen efficiency evaluation indices to a closed interval [0, 1] for comprehensive evaluation. The formula is:

Yij = (Xij - Xjmin) / (Xjmax - Xjmin)

where Yij represents the subordinate function value of the j-th evaluation index for the i-th cultivar, Xij represents the measured value of the j-th evaluation index for the i-th cultivar, Xjmin represents the minimum value of the j-th evaluation index across all cultivars, Xjmax represents the maximum value of the j-th evaluation index across all cultivars, i represents a specific cultivar, and j represents a specific evaluation index.

Weights were calculated using the objective weighting method with the formula:

Ej = Cj / ΣCj

where Ej represents the weight of the j-th evaluation index, and Cj represents the coefficient of variation of the j-th evaluation index. The comprehensive nitrogen efficiency value P = [Σ(Yij × Ej)] / 5.

1.4 Data Analysis Methods

Data were analyzed using Microsoft Excel 2007, SPSS 19.0, and HemI 1.0 heatmap illustrator software. Comprehensive nitrogen efficiency values were calculated using the subordinate function method. Hierarchical heatmap analysis was performed using squared Euclidean distance fitting to reflect similarities and differences among cultivars through color gradients and similarity patterns.

2.1 Variation Characteristics of Rice Seedling Traits Under Different Nitrogen Forms

The coefficient of variation measures the degree of variation in traits among cultivars; a larger coefficient indicates more significant differences in nitrogen absorption and accumulation among cultivars. Under both NH -N and NO -N supply conditions, rice trait indices exhibited certain variability with substantial differences in variation ranges (Table 2).

Under NH -N culture conditions, the coefficients of variation for rice trait indices ranged from 0.36 to 0.80, with shoot nitrogen content showing the smallest variation (0.36) and root nitrogen content showing the largest (0.80). Under NO -N culture conditions, coefficients of variation ranged from 0.33 to 0.74, with root nitrogen accumulation showing the smallest variation (0.33) and shoot biomass, root biomass, and root nitrogen content showing the largest (all 0.74).

Under NH -N culture conditions, the coefficients of variation for whole-plant biomass, shoot biomass, root biomass, and root nitrogen content all exceeded 0.70, indicating these traits effectively demonstrate differences among rice cultivars. The coefficients of variation for whole-plant nitrogen content, shoot nitrogen content, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation were below 0.70. Additionally, the coefficients of variation for root nitrogen content, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation were greater under NH -N than under NO -N, suggesting that different rice cultivars exhibit different phenotypes under NH -N versus NO -N culture, likely related to different mechanisms of nitrogen absorption and utilization.

Under NH -N supply conditions, all nutritional parameter values (except root biomass) were greater than those under NO -N supply conditions. Whole-plant biomass, shoot biomass, whole-plant nitrogen content, shoot nitrogen content, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation showed significant differences ($P < 0.05$), while shoot biomass, whole-plant nitrogen content, shoot nitrogen content, whole-plant nitrogen accumulation, shoot nitrogen accumulation, and root nitrogen accumulation showed extremely significant differences ($P < 0.01$).

2.2 Factor Analysis of Rice Seedling Nutritional Traits Under Different Nitrogen Forms

Factor analysis was performed on nine nutritional parameters of rice seedlings under NH -N and NO -N supply conditions to calculate eigenvalues and cumulative contribution rates. The total variance explanation for rice nutritional traits under NH -N and NO -N culture yielded retained principal component loading matrices.

Through factor analysis (using a critical cumulative proportion value of 0.85 for eigenvalues) and loading matrices, two principal components were identified under both NH -N and NO -N culture conditions, with cumulative contribution rates of 93.78% and 93.46%, respectively. Under NH -N culture, the first principal component accounted for 68.94% of variance and the second for 24.83%. The first principal component was primarily determined by whole-plant nitrogen accumulation, shoot nitrogen accumulation, whole-plant biomass, shoot biomass, root biomass, and root nitrogen accumulation, showing strong correlations and mainly reflecting plant biomass and nitrogen accumulation indicators. Under NO -N culture, the first principal component accounted for 72.61% of variance and the second for 20.85%. The first principal component was primarily determined by whole-plant nitrogen accumulation, shoot nitrogen accumulation, root nitrogen accumulation, whole-plant biomass, shoot biomass, and root biomass, which closely resembles the determination of principal components under NH -N culture. Under both NH -N and NO -N culture, the second principal component was determined by nitrogen content. The contribution rates of each principal component indicate that biomass and nitrogen accumulation indicators have the greatest influence on rice nitrogen absorption and accumulation under both nitrogen forms (Tables 3 and 4).

2.3 Comprehensive Nitrogen Efficiency Values and Heatmap Analysis of Rice Seedlings Under Different Nitrogen Forms

Based on variation characteristics and factor analysis of nitrogen nutritional parameters at the seedling stage, whole-plant biomass, shoot biomass, root biomass, and shoot nitrogen accumulation were identified as comprehensive evaluation indices for nitrogen efficiency at the seedling stage. Absolute evaluation index values were transformed to relative values, and comprehensive nitrogen efficiency values for different rice cultivars were obtained through composite calculation (Table 5).

Under NH -N culture conditions, the comprehensive nitrogen efficiency values ranged from 0.21 to 1.14, with a mean of 0.51. 'Fengliangyou' showed the minimum value (0.21), while 'Liangyou 766' showed the maximum (1.14). Under NO -N culture conditions, the comprehensive nitrogen efficiency values ranged from 0.22 to 1.12, with a mean of 0.48. 'Zhendao 14' showed the minimum value (0.22), while 'Yongyou 9' showed the maximum (1.12).

Hierarchical heatmap analysis was performed using the comprehensive nitrogen efficiency values of 55 rice cultivars at the seedling stage. Color variations visually represented data magnitude, with color depth gradients and similarity patterns reflecting data similarities and differences among cultivars (Figure 1 [Figure 1: see original paper]). The comprehensive nitrogen efficiency values under NH -N culture were generally higher than those under NO -N culture. 'Fengliangyou' showed the lowest average comprehensive nitrogen efficiency values under both NH -N and NO -N conditions (0.21 and 0.24, respectively), while 'Yongyou 9' showed the highest (1.08 and 1.12, respectively).

Comprehensive analysis of nitrogen efficiency values under NH -N and NO -N conditions through Euclidean distance-based cluster analysis divided the tested rice cultivars into three groups. Group 1 comprised nitrogen-efficient cultivars (6 cultivars, 10.9% of total) with average comprehensive nitrogen efficiency values ranging from 0.95 to 1.10. Group 2 comprised nitrogen-moderately efficient cultivars (15 cultivars, 27.3%) with values ranging from 0.60 to 0.86. Group 3 comprised nitrogen-inefficient cultivars (34 cultivars, 61.8%) with values ranging from 0.22 to 0.54.

Discussion

Screening and breeding nitrogen-efficient rice cultivars represents an important pathway for improving nitrogen use efficiency and reducing nitrogen loss. Significant genotypic differences exist in nitrogen utilization efficiency among different rice cultivars. Root architecture is one of the key factors affecting rice nitrogen absorption. Studies have shown that nitrogen-efficient rice exhibits significantly or extremely significantly greater root dry weight, root volume, total absorption surface area, active absorption surface area, and active absorption surface area ratio compared to nitrogen-inefficient rice. This may be because nitrogen-efficient rice possesses stronger root initiation capacity, resulting in greater whole-root dry weight and root volume, thereby enhancing nitrogen absorption capacity. Significant differences also exist in enzymatic metabolic activities among different rice genotypes. Higher nitrogen absorption capacity and nitrogen content in rice correspond to higher nitrate reductase activity, which facilitates nitrogen assimilation and utilization. The results of this study demonstrate significant differences in nitrogen efficiency values among 55 rice cultivars under uniform nitrogen supply levels, likely related to superior root morphological systems in nitrogen-efficient cultivars that enable greater nitrogen absorption and dry matter production.

Rice nitrogen use efficiency depends not only on cultivar genetic characteristics but also on nitrogen form. Rice is an ammonium-preferring crop; at the same nitrogen supply level, ammonium nitrogen culture promotes greater nitrogen accumulation than nitrate nitrogen culture. The results indicate that comprehensive nitrogen efficiency values are higher in NH -N systems than in NO -N systems, suggesting that rice cultured with NH -N possesses superior nitrogen absorption and accumulation capacity. This may be because rice exhibits higher plasma membrane proton pump activity in roots under NH -N nutrition than under NO -N nutrition, resulting in stronger nitrogen absorption capacity. After entering plant tissues, NH -N can be rapidly assimilated in root cells or transported to shoots for assimilation in leaves. NH -N combines with organic acids in plant cells to form amino acids or amides, which then synthesize proteins. NO -N must be reduced to NH after entering the plant before being assimilated into amino acids and proteins for participation in rice nitrogen metabolism.

Soil microorganisms can utilize oxygen secreted by rice roots to oxidize NH -N to NO -N, placing rice in mixed ammonium-nitrate nutrition that promotes NH -N absorption, intracellular NH -N accumulation and metabolism, and enhances glutamine synthetase and nitrate reductase activities, thereby promoting NH -N assimilation and increasing nitrogen accumulation in plants.

Most studies on nitrogen-efficient resource evaluation and screening focus on the post-heading period to yield formation, using yield as the most intuitive indicator for nitrogen efficiency evaluation and screening. However, this approach suffers from long cycles, heavy workload, and susceptibility to climate, soil environment, and disease factors. Singh et al. demonstrated that 75% of yield variation in rice is caused by nutrient absorption efficiency, with nitrogen-efficient rice cultivars generally maintaining higher absorption and utilization efficiency and nitrogen accumulation. Furthermore, nitrogen use efficiency is closely related to crop growth periods.

References

[13] Vijayalakshmi P, Vishnukiran T, Kumari B R, et al. Biochemical and physiological characterization for nitrogen use efficiency in aromatic rice genotypes[J]. Field Crops Research, 2015, 179: 132-143.

[14] Xu G H, Fan X R, Miller A J. Plant nitrogen assimilation and use efficiency[J]. Annual Review of Plant Biology, 2012, 63(3): 153-182.

[15] Zhang Y L, Fan J B, Duan Y H, et al. Variation of nitrogen use efficiency of rice different in genotype and its evaluation[J]. Acta Pedologica Sinica, 2008, 45(2): 267-273.

[16] Huang L F, Dong F R, Huo Z Y, et al. Effects of nitrogen levels on dry matter accumulation and distribution in rice genotype with different nitrogen use efficiencies[J]. Journal of Nuclear Agricultural Sciences, 2012, 26(9): 1290-1297.

[17] Zhang C M, Xu Y H, Zhao H J, et al. Effects of different nitrogen forms on nitrogen uptake and root growth of rice at the seedling stage[J]. Journal of Nanjing Agricultural University, 2011, 34(3): 72-76.

[18] Duan Y H, Zhang Y L, Shen Q R, et al. Effect of partial replacement of NH by NO on nitrogen uptake and utilization by different genotypes of rice at the seedling stage[J]. Plant Nutrition and Fertilizer Science, 2005, 11(2): 160-165.

[19] Mao D R. The Methods of Plant Nutrition Research[M]. Beijing: Beijing Agricultural University Press, 1994.

[20] Wang H Z. Study on drought resistance and screening identification indexes of drought resistance in rice[D]. Chengdu: Sichuan Agricultural University, 2007.

[21] Chen W R, Zeng W W, Li Y X, et al. The physiological responds of highbush blueberry to drought stress and the comprehensive evaluation on their drought resistance capacity[J]. Acta Horticulturae Sinica, 2012, 39(4): 637-646.

[22] Li Q, Luo Y H, Tan J, et al. Indexes screening and comprehensive evaluation of low nitrogen tolerance of hybrid maize cultivars at seedling stage[J]. Chinese Journal of Eco-Agriculture, 2014, 22(10): 1190-1199.

[23] Xu Y X, Li X Y, Yang W G, et al. Evaluation of drought resistance in maize seedling emergence stage using principal components analysis and cluster analysis[J]. Crops, 2014(3): 54-57.

[24] Huang Y, Zhang B, Wu X J, et al. Variety screening for mung bean paste production based on principal component analysis[J]. Food Science, 2012, 33(13): 104-107.

[25] Hu B L, Li X, Wan Y, et al. Index screening and comprehensive evaluation of phenotypic traits of low nitrogen tolerance using BILs population derived from Dongxiang wild rice (Oryza rufipogon Griff.)[J]. Chinese Journal of Applied Ecology, 2015, 26(8): 2346-2352.

[26] Xu F X, Xiong H, Xie R, et al. Advance of rice fertilizer-nitrogen use efficiency[J]. Plant Nutrition and Fertilizer Science, 2009, 15(5): 1215-1225.

[27] Wei H Y, Zhang H C, Zhang S F, et al. Root morphological and physiological characteristics in rice genotypes with different N use efficiencies[J]. Acta Agronomica Sinica, 2008, 34(3): 429-436.

[28] Abreu M H, Pereira R, Buschmann A H, et al. Nitrogen uptake responses of Gracilaria vermiculophylla (Ohmi) Papenfuss under combined and single addition of nitrate and ammonium[J]. Journal of Experimental Marine Biology and Ecology, 2011, 407(2): 190-199.

[29] Zhang Y L, Shen Q R, Duan Y H. Physiological effects of different nitrogen forms on rice[J]. Journal of Nanjing Agricultural University, 2004, 27(2): 130-135.

[30] Li S M, Shi W M. Effect of nitrogen form on root morphology and nitrogen absorption efficiency of two cultivars of rice[J]. Soils, 2007, 39(4): 589-593.

[31] Sun Y J, Ma J, Sun Y Y, et al. The effects of different water and nitrogen managements on yield and nitrogen use efficiency in hybrid rice of China[J]. Field Crops Research, 2012, 127: 85-98.

[32] Samonte S O P, Wilson L T, Medley J C, et al. Nitrogen utilization efficiency: Relationships with grain yield, grain protein, and yield-related traits in rice[J].

Agronomy Journal, 2006, 98(1): 168-176.

[33] Zhang Y L, Dong Y Y, Shen Q R, et al. Characteristics of NH and NO uptake by rice of different genotypes[J]. Acta Pedologica Sinica, 2004, 41(6): 918-923.

[34] Hirel B, Limami A M. Prospects for improving nitrogen use efficiency: Insights given by ¹ N-labelling experiments[J]. Phytochemistry Reviews, 2002, 2(1/2): 133-144.

[35] Wang S W. Response of the plasma membrane H -ATPase of rice seedling to ammonium and nitrate nutrition[D]. Nanjing: Nanjing Agricultural University, 2008.

[36] Singh U, Ladha J K, Castillo E G, et al. Genotypic variation in nitrogen use efficiency in medium- and long-duration rice[J]. Field Crops Research, 1998, 58(1): 35-53.

[37] Pei X X, Wang J A, Dang J Y, et al. Study on screening index for low nitrogen tolerant wheat genotype[J]. Plant Nutrition and Fertilizer Science, 2007, 13(1): 93-98.

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