Establishment and Application of Critical Nitrogen Dilution Curve for Rice Based on Leaf Dry Matter

Lijuan Song 1,2, Shu Wang 1,* and Wanjun Ye 2

1 College of Agronomy, Shenyang Agricultural University, Shenyang 110866, China; songlijuan-2007@163.com
2 Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China; nmslj_2002@163.com
* Correspondence: swang123@syau.edu.cn; Tel.: +86-24-88487135

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Abstract: In order to investigate the feasibility of using rice leaf critical nitrogen concentration as a nitrogen nutrition diagnosis index, a three-year positioning experiment with large-spike rice cultivar (Wuyoudao4) and multiple-spike rice cultivar (Songjing9) under five nitrogen levels (0, 60, 120, 180, and 240 kg·ha\(^{-1}\)) was conducted. A critical nitrogen dilution curve and a nitrogen nutrition index (NNI) of rice leaf dry matter were constructed for Wuyoudao4 \((N_c = 1.96L_{DM}^{-0.56}, R^2 = 0.87, \text{NNI was between 0.6–1.26})\) and Songjing9 \((N_c = 1.99L_{DM}^{-0.44}, R^2 = 0.94, \text{NNI was between 0.64–1.29})\). The relationship between dry matter and nitrogen concentration of rice leaves was a negative power function, and the model had good stability over the three years. The developed critical nitrogen concentration dilution curve, based on leaf dry matter, was able to diagnose nitrogen nutrition in rice efficiently. The model established in this study could be used to directly regulate and control the nitrogen nutrition of rice leaves.

Keywords: rice; leaf; critical nitrogen dilution curve; nitrogen nutrition index

1. Introduction

Heilongjiang Province is the main region for japonica rice production and is a significant commercial food base for China. The yields of japonica rice from Heilongjiang can reach up to 281,950,000 tons, ranking first in China. In order to improve people’s lives, we need to improve the yield and quality of rice. Compared to conventional rice, super rice has a high biological yield and strong growth potential, which is appropriate for high yield potential of rice in a high nitrogen environment [1]. The high-quality aromatic japonica rice, Wuyoudao4, is prone to lodging and yield reduction due to improper water and fertilizer management. In order to fully meet the growth needs of rice, excessive nitrogen fertilizer is applied [2]. Although proper nitrogen fertilizer application is necessary to improve rice yield, excessive nitrogen fertilizer application leads to a high cost of production. Other undesirable effects include environmental pollution and decline in the quality of rice, all of which negatively affect sustainable agricultural development [3]. Consequently, to realize rational fertilization of rice scientifically, it is necessary to find the critical point of nitrogen demand that can maximize rice yield with the lowest amount of nitrogen applied.

Nitrogen nutrition diagnosis is a key technology involving precise application of nitrogen fertilizer [4]. Presently, nitrogen nutrition diagnosis mainly involves nitrate rapid diagnosis, SPAD (Soil Plant Analysis Development) meter rapid diagnosis, spectral diagnosis, and remote sensing technology [5,6]. However, the results are unstable during the period when the crop is in luxury absorption [7]. Greenwood et al. established the critical nitrogen concentration dilution curve \((N_c = aw^{-b})\) for \(C_3\) and \(C_4\) plants in order to overcome the measurement error caused by excessive nitrogen concentration in plants [8]. Based on plant dry matter and plant nitrogen concentration, the
critical nitrogen concentration dilution curve is developed to find out the lowest nitrogen concentration when the aboveground dry matter reaches the maximum growth rate. Presently, this model has been applied to rape, cotton, potato, wheat, and rice crops [9–13]. Specifically, for rice, the critical nitrogen model is based on the analysis of the entire plant; the curve changes as a result of the differences in climate, environment, and genetics. Mostly, this occurs when the plant is subjected to stress [13–16], this changes the distribution of dry matter; thus, affecting the establishment of the curve [17]. Consequently, it is important to study the dilution curve of critical nitrogen concentration in different crop organs.

Nitrogen accumulation is a crucial factor affecting the yield potential of rice. As rice grows, leaves facilitate photosynthesis, and the dry matter of leaves is an important indicator of growth potential, the light energy utilization rate, and the yield of crop [18]. Leaves are sensitive to changes in nitrogen nutrition within the environment. Ordinarily, dry matter and nitrogen concentration in leaves of rice increase along with the increase of nitrogen application. When nitrogen application reaches a certain level, the dry matter of leaves stabilizes, while the nitrogen concentration continues to increase. However, with the extension of the rice growth stage, dry matter accumulation of leaves increases, and nitrogen concentration in the leaves decreases [19]; therefore, the level of nitrogen in leaves is an important indicator that can be used to determine plant growth status and estimate yield. The reason for the drop in nitrogen concentration of the rice leaves is due to the shading phenomenon. In the process of crop growth, the leaves keep growing and the number and area of leaves keep increasing. The number and degree of shading in lower leaves continues to increase, while the upper leaves generally receive more sunlight than that of lower leaves [20]. This leads to nitrogen dilution [21,22], and provides a theoretical basis for establishing a critical nitrogen concentration dilution curve using the dry matter of rice leaves. In the study of rice, researchers across different rice-growing regions and countries have initiated studies to determine critical nitrogen concentrations in varying conditions [17,21]. In the present research, the power function curve equation of the critical nitrogen concentration of rice and dry matter mass was obtained.

In this study, the critical nitrogen concentration curve model was used to analyze different genotypes of high-quality rice leaves and determine the critical nitrogen concentration. The goal was to explore the rice leaf, as well as the applicability of the critical nitrogen concentration dilution curve model and nitrogen nutrition index (NNI) to evaluate the nitrogen nutrition of rice.

2. Materials and Methods

2.1. Experimental Design

Field experiments were carried out with five N rates (0, 60, 120, 180, and 240 kg N ha$^{-1}$, represented by N0, N60, N120, N180, and N240, respectively) using two japonica rice (*Oryza sativa* L.) cultivars, Wuyoudao4 and Songjing9, in the Heilongjiang Province of Northeast China, as detailed in Table 1. Data used to develop the $N_c$ dilution curve was drawn from two experiments conducted in 2016 and 2017 that examined five N fertilizer rates, ranging from zero to non-limiting amounts of N. The data used to validate the $N_c$ dilution curve came from an independent experiment conducted in 2018 with five levels of N fertilizer, ranging from N-limiting to non-limiting amounts of N.
Table 1. Basic information about three experiments.

| Experiment No. | Transplanting/ Harvesting Date | Location | Cultivar | N Rate (kg ha\(^{-1}\)) | Sampling Stage | Sampling Date | Soil Characteristics |
|---------------|---------------------------------|----------|----------|--------------------------|----------------|---------------|---------------------|
| Experiment 1 in 2016 | 20-May/25-Sep | Wuchang (44°92' N, 127°15' E) | Wuyoudao4 Songjing9 | N(0) N\(_{60}(60)\) N\(_{120}(120)\) N\(_{180}(180)\) N\(_{240}(240)\) | Active tillering Panicle initiation Stem elongation Booting Heading | 13-Jun 27-Jun 8-Jul 23-Jul 13-Aug | Soil type = Brunisolic Soil pH = 6.59 Total P = 2.15g kg\(^{-1}\) Total K = 17.5g kg\(^{-1}\) Available N = 114ppm Available P = 37.8ppm Available K = 156ppm |
| Experiment 2 in 2017 | 18-May/24-Sep | Wuchang (44°92' N, 127°15' E) | Wuyoudao4 Songjing9 | N(0) N\(_{60}(60)\) N\(_{120}(120)\) N\(_{180}(180)\) N\(_{240}(240)\) N\(_{300}(300)\) | Active tillering Panicle initiation Stem elongation Booting Heading Active tillering Panicle initiation Stem elongation Booting Heading | 10-Jun 24-Jun 22-Jul 6-Aug 15-Jun 28-Jun | Same as above |
| Experiment 3 in 2018 | 22-May/25-Sep | Wuchang (44°92' N, 127°15' E) | Wuyoudao4 Songjing9 | N(0) N\(_{60}(60)\) N\(_{120}(120)\) N\(_{180}(180)\) N\(_{240}(240)\) | Active tillering Panicle initiation Stem elongation Booting Heading | 9-Jul 21-Jul 8-Aug | Same as above |
2.2. Plant Sampling and Determination of N Content in Tissues

2.2.1. Plant Sampling

Samples were drawn from five separate hills and were used to determine the plot rice growth. Five sampling dates occurred during each experiment year. The sampling process took place from active tillering to heading (before the onset of flowering) at intervals of 10–12 days, starting from 16 and 18 days after transplanting (DAT) in 2016 and in 2017, respectively. The sampling dates are presented in Table 1. Whole plants were manually uprooted and samples were divided into leaf blades (leaves) and culms plus sheaths (stems); fresh plants were separated into different leaves (green leaves and the rest of leaves, other than the green leaves).

2.2.2. Determination of Biomass and N Content in Leaves and Determination of Yields

Shoot biomass (t ha\(^{-1}\)) was determined by severing five plants from each plot at ground level on each sampling date. Fresh plants were separated into leaf blades (leaves) and culms plus sheaths (stems). Leaf dry matter (L\(_{\text{DM}}\)) was determined after each sample was oven-dried at 80 °C for 48 h. After entering the mature stage, the total number of panicles in the field of each community was investigated. At the same time, 30 plants were selected for indoor seed examination. Important indicators, such as grain number per panicles, grain weight per panicles, and 1000-grain weight was observed and recorded. The leaf samples were subsequently grounded to a powder to pass through a 1-mm sieve in a Wiley mill and stored at room temperature until further chemical analysis. Total N concentration in leaf samples was determined by using the micro-Kjeldahl method [23].

2.2.3. Plant N Accumulation

Leaf N accumulation (L\(_{\text{NA}}\)) was obtained as summed products of the leaf dry matter of different leaves by the N contents in the corresponding leaf on dry matter (DM) basis.

\[
L_{\text{NA}} = (L_{\text{GDM}}L_{\text{GN}} + L_{\text{RDM}}L_{\text{RN}})/100
\]  

where L\(_{\text{GDM}}\) is dry matter (t ha\(^{-1}\)) of green leaves, and L\(_{\text{N}}\) is N concentration of corresponding leaf, respectively; L\(_{\text{RDM}}\) is rest of leaves other than the green leaves.

2.3. Data Analysis

The concept of a N\(_{\text{c}}\) dilution curve [24] based on whole-plant N concentration was developed for tall fescue by Lemaire and Salette [25] and is represented by a power equation:

\[
N_{\text{c}} = aW^{-b}
\]  

where \(W\) is the aerial biomass expressed in t ha\(^{-1}\), \(N_{\text{c}}\) is the N concentration in shoots expressed as a percentage of shoot dry matter, and \(a\) and \(b\) are estimated parameters. N concentration in shoots expressed as a percentage of shoot dry matter, while \(a\) and \(b\) are estimated parameters. N concentration in the shoot biomass (t ha\(^{-1}\)) is represented by the parameter \(a\) and the coefficient of dilution describing the relationship between N concentration and shoot biomass is represented by the parameter \(b\). The curve defined by Equation (2) differentiates N status into three categories for plant growth: N limiting (below the curve), non-N limiting (above the curve), and optimal N concentration (on the curve). Notably, during the early stages of growth (when shoot biomass <1 t ha\(^{-1}\)), \(N_{\text{c}}\) is a constant value due to the small decline of N\(_{\text{c}}\) with increasing shoot biomass and the absence of competition for light in well-spaced plants, resulting in a constant N concentration value [25,26].

The data was analyzed to determine the N\(_{\text{c}}\) concentration using a method developed by Justes et al. [24]. Analysis of variance (ANOVA) was applied to compare the amount of leaf dry matter under different N rates and the corresponding N concentrations at each sampling date and year using
GLM (Generalized Linear Model) procedures in SPSS-22 software package (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test at 95% level of significance was used to assess the difference between treatment means in order to calculate the critical value at the intersection of a vertical line and an oblique line. The regression between leaf dry matter (t ha\(^{-1}\)) and N concentration (%\(L_{DM}\)) was conducted using Microsoft Excel (Microsoft Cooperation, Redmond, WA, USA).

The \(N_c\) dilution curve was determined by identifying the data points where N does not limit growth (N-limiting) or is not in excess (non-N-limiting) using the experimental data from 2016 and 2017. N-limiting growth treatment is defined as a treatment of N, which leads to a significant increase in leaf dry matter, while non-N-limiting growth treatment is defined as a treatment of N, which does not lead to an increase in leaf dry matter, but significantly increases N concentration. If, on the same measurement date, statistical analysis distinguished at least one set of N-limiting and non-N limiting data points, this series of data was used to define the critical N dilution curve (Figure 1). Series of data, which presented only N-limiting or non-N-limiting data points, were used to validate the curve [24]. These data points were used to determine the relationship between N concentration and leaf dry matter using an allometric function. The critical curve is validated firstly by applying data points that have not been retained for establishing the parameters of the allometric function in 2016 and 2017, and then with independent data set from 2018.

Figure 1. Critical nitrogen concentration curve.

The verification of the model adopts the internationally accepted standard error Root Mean Square Error (RMSE) and Normalized Root Mean Square Error (n-RMSE) of regression estimation, as well as a 1:1 histogram between the simulated value and the measured value in order to detect the fitting degree and reliability of the model. The calculation formulas of RMSE and n-RMSE are as follows:

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (s_i - m_i)^2}{n}}
\]

\[
n\text{-RMSE} = \frac{\text{RMSE}}{\text{S}} \times 100\%
\]

where, \(s_i\): simulation value; \(m_i\): measured value; \(n\): data format; \(S\): average value of measured data.

The smaller the RMSE [27] value the better the consistency between simulation value and real value. The smaller deviation is, i.e., the higher the accuracy of the model. n-RMSE [28] has no limitations based on units used and can be utilized to compare the model stability of data with different units. Generally, a model with a n-RMSE value < 10% is considered to be extremely stable, 10% <
n-RMSE < 20% is considered stable, 20% < n-RMSE < 30% is considered generally stable, and an n-RMSE > 30% has poor stability [29].

Data points were solely selected from non-N-limiting treatments (N180) for the determination of the upper limit curve (N_{\text{max}}), while data points from N-limiting treatments for which N application zero (N0 check plots) were used to determine lower limit curve (N_{\text{min}}). Further, the N nutrition index (NNI) of the crop at each sampling date was determined by dividing the total N concentration of the leaf (N_t) by N_c, according to previous reports on potato and corn, as shown in Equation (4).

\[ \text{NNI} = \frac{N_t}{N_c} \] (4)

3. Results

3.1. Leaf Dry Matter and N Concentration

N application rates exhibited a significant effect on leaf dry matter throughout the growth period, ranging from the minimum value of 0.16 t ha\(^{-1}\) (N0) for Wuyoudao4 to a maximum value of 3.15 t ha\(^{-1}\) (N240) for Songjing9, both observed during 2017. Leaf dry matter continuously increased as N application rates increased along the sampling dates for both varieties during each year. Leaf dry matter also increased as N application increased from N0 to N240 for both cultivars; however, there was no statistical difference between N180 to N240 in all cases (Figure 2).

Figure 2. Changes of rice leaf dry matter (t ha\(^{-1}\)) with time (days after transplantation) under different N application rates in experiments conducted during 2016 and 2017. WYD-4 and SJ-9 refer to Wuyoudao4 and Songjing9, respectively, the same as below.

The maximum variation in N concentration of both cultivars was observed on day 15 after transplantation in 2016, while the minimum variation in N concentration was observed on day 75 after transplantation in 2017. N concentration ranged from 0.85% to 4.88% for Wuyoudao4 and 0.85% to 4.16% for Songjing9 (Figure 3).
The maximum variation in N concentration of both cultivars was observed on day 15 after transplantation in 2016, while the minimum variation in N concentration was observed on day 75 after transplantation in 2017.

N concentration ranged from 0.85% to 4.88% for Wuyoudao4 and 0.85% to 4.16% for Songjing9 (Figure 3).

Figure 3. Changes of nitrogen (N) concentration (%LDM) with time (days after transplantation) for rice under different N application rates in experiments conducted during 2016 and 2017.

3.2. Determination of Dilution Curves for Nc Concentration

Data points for each sampling date from the time of tillering to the heading period for both cultivars were used to determine the Nc points by following the computation method of Justes et al. (1994) [24]. Twenty data points between 0.16 t ha\(^{-1}\) and 3.15 t ha\(^{-1}\) of leaf dry matter allowed the calculation of the theoretical Nc points. The Nc points were determined by intercept between the vertical and oblique lines fitted through the data points on each sampling date for Wuyoudao4 and Songjing9. Declining trends of the Nc values were observed in both cultivars as critical leaf dry matter increased, which were fitted as Equations (5) and (6) with determination coefficients of 0.87 and 0.94 for Wuyoudao4 and Songjing9, respectively (Figure 4). The two curves showed no statistical differences when tested based on Hahn’s (1997) methodology.

\[
N_c = 1.96 L_{DM}^{-0.56}, \, R^2 = 0.87 \tag{5}
\]

\[
N_c = 1.99 L_{DM}^{-0.44}, \, R^2 = 0.94 \tag{6}
\]
3.3. Validation of the $N_c$ Dilution Curve

The $N_c$ dilution curve was dually validated for N-limiting and non-N-limiting situations within the range for which it was established. Data points not engaged for establishing the parameters of the allometric function were used for partial validation of $N_c$ dilution curve. The significantly varying ($P \leq 0.01$) data points of $L_{DM}$ were selected from each treatment (N-limiting or non-N-limiting) for each sampling date in 2018. Independent data points from an experiment conducted in different pedoclimatic conditions were used for comprehensive validation of the $N_c$ dilution curve. In general, the new $N_c$ dilution curve on $L_{DM}$ basis well discriminated the limiting and non-N-limiting conditions of rice crop grown under varied N rates. All data points from N-limiting treatments were close to or lower than $N_c$ dilution curve, whereas those from non-N-limiting treatments were close to or above the $N_c$ dilution curve (Figure 5).

The verification of the above model showed that the relationship between $N_c$ simulation values and measured values of the two varieties of rice could directly be displayed by a 1:1 histogram. An RMSE of 0.31 and an n-RMSE of 13.07% were calculated for Wuyoudao4, according to the formula. An
RMSE of 0.37 and an n-RMSE of 15.89% was calculated for Songjing9, indicating that the model is highly stable; therefore, the critical nitrogen concentration dilution curve of rice leaves can be used for nitrogen nutrition diagnosis of rice leaves (Figure 6).

Figure 6. Calibration $N_c$ dilution curve of leaf dry matter of rice ($r_{0.01} = 0.765$). The data of N120 and N180 were selected for verification.

3.4. Effect of Different Nitrogen Application Levels on Nitrogen Nutrition Index (NNI) in Rice Leaves

Based on the evaluation system of nitrogen nutrition index (NNI), the nitrogen nutrition status of leaves was quantified (Figure 7). The NNI is divided according to the size of the value “1”. For the Wuyoudao4 rice variety, a state of nitrogen nutrient deficiency occurred when the NNI < 1 (the N application rate was less than 120 kg ha$^{-1}$); an NNI of 1 occurred when nitrogen nutrition was moderate (N application rate was 120 kg ha$^{-1}$), and when the amount of nitrogen applied exceeded 180 kg ha$^{-1}$, the NNI was greater than 1 and nitrogen was in excess. For the Songjing9 rice variety, the NNI was less than 1 when the amount of N application was less than 180 kg ha$^{-1}$, the NNI = 1 when nitrogen nutrition was moderate (N application was 180 kg ha$^{-1}$), and when the amount of N applied exceeded 180kg ha$^{-1}$, the NNI > 1, which resulted in excess nitrogen nutrition.

Figure 7. Changes of nitrogen nutrition index (NNI) with time (days after transplanting) for rice under different N application rates in experiments conducted during 2016 and 2017.

4. Discussion

The critical nitrogen concentration dilution curve is an important method for crop nitrogen nutrition diagnosis in most countries. It is used to diagnose crop nitrogen deficiency and to determine
the nitrogen requirements of a crop. In order to determine and understand yield composition and the quality of growth formation in crops, chlorometer readings (SPAD values) and nutritional diagnoses of plants were carried out. The SPAD technique is determined by leaf color and was appropriate and easy to use. It is very sensitive to the changes in ammonia and is equally sensitive to nutritional changes.

In this study, the curve equation of critical nitrogen change in rice was established using the relationship between the leaf dry matter and nitrogen concentration of the fractionated rice ($N_c = 1.96L_{DM}^{-0.56}$ and $N_c = 1.99 L_{DM}^{-0.44}$ for Wuyoudao4 and Songjing9, respectively).

The determination coefficients of the equation were 0.87 and 0.94 for Wuyoudao4 and Songjing9, respectively. The fitting degree was significant for the species. Compared to the parameters of the curve equation obtained by Sheehy et al. (Table 2). The blade critical nitrogen variation curve equation parameter values a and b varied greatly in this study. It was observed that the curve equation of critical nitrogen concentration change in rice is lower than previous research results; additionally, predecessors’ study results indicated an uptick in japonica rice nitrogen uptake. Table 2 showed that the reference curve on stem dry matter (SDM) basis of japonica rice (2.17) by Ata-Ul-Karim et al. [30] was lower than the reference curve on whole plant dry matter basis of Indica rice in tropics (5.20) by Sheehy et al. [21]. It was also lower than the curves developed with japonica rice on whole plant dry matter basis (3.53) by Ata-Ul-Karim et al. [22], and $L_{DM}$ basis (3.76) by Yao et al. [13]. The parameter a of $N_c$ dilution curve on $L_{DM}$ basis with japonica rice developed in the present study (1.96,1.99) was lower than the reference curve on whole plant dry matter basis of japonica rice in tropics (2.77) by Huang et al. [31]. The analysis suggests that this is mainly related to the asynchronous accumulation of leaf dry matter and plant dry matter. When leaf dry matter reaches 1 t ha$^{-1}$, plant dry matter is already greater than 1 t ha$^{-1}$, and nitrogen concentration dilution has occurred. As a result, the “a” value of the dilution curve based on the critical nitrogen concentration of leaf dry matter is low. The relationships between plant dry matter (PDM) based, with leaf area index (LAI), $L_{DM}$, and SDM based N nutrition index, accumulated N deficit, and N requirement indicated that leaf based approaches could be used as substitutes for PDM approach [32]. Moreover, the real-time, quick, and non-destructive field methods (chlorophyll meter, hyper-spectral meter, remote sensing, and digital photography) generally monitor N concentration at single leaf or on canopy basis, instead of whole-plant basis [14,15]. Besides, the leaf is a major photosynthetic organ, and highly responsive to N fertilization [33,34]. Thus, it appears that the leaf based $N_c$ dilution curve could be more useful for assessing N status in crop plants.

**Table 2. Summary of critical nitrogen concentrations.**

| Region         | Cultivar                                      | Model                          | Researcher                  |
|----------------|-----------------------------------------------|--------------------------------|-----------------------------|
| Torrid zone    | Cultivars in different regions                | $N_c = 5.2 PDM^{-0.5}$         | Sheehy et al. [21]          |
| South China    | Zhongjiazao17, Tanliangyou83                  | Early rice: $N_c = 3.37 PDM^{-0.44}$ | He et al. [35]              |
|                | Tianyouhuanzhan, Yueyou9113, Xiangyou186 etc. | Late rice: $N_c = 3.69 PDM^{-0.34}$ | Wang et al. [36]            |
| South China    | Nanjing46, Nangan48, Wuyujing24 etc. Yiangyou1, | $N_c = 3.33 PDM^{-0.26}$     | Yi et al. [37]              |
| South China    | Chaoyouqianhao etc. Jinongsimiao, Yuenongsimiao etc. | Hybrid rice: $N_c = 3.36 PDM^{-0.31}$ | Yang et al. [38]            |
| South China    | Xishu63, Hang43                               | Conventional rice: $N_c = 2.96 PDM^{-0.25}$ | Zhong [19]                 |
| South China    | Hang43                                        | 2013-Hang43: $N_c = 5.31 PDM^{-0.5}$ | Huang et al. [31]           |
|                |                                               | 2015-Hang43: $N_c = 5.38 PDM^{-0.49}$ | Ata-Ul-Karim et al. [22]    |
| Northern China | Kongyu131, Longqing31                          | $N_c = 2.77 PDM^{-0.34}$     | Yao et al. [13]             |
| South China    | Lingxiangyou18, Wuxiangjing14 etc.             | $N_c = 3.53 PDM^{-0.28}$     | Ata-Ul-Karim et al [30]     |
| South China    | Wuxiangjing14 etc.                            | $N_c = 3.76 L_{DM}^{-0.22}$ |                             |
| South China    | Lingxiangyou18, Wuxiangjing14 etc.             | $N_c = 2.17 SDM^{-0.27}$ |                             |
Different analytical parameters may arise from the fact that the equation constructed by Sheehy et al. [21] is a result of integrating rice varieties from different countries. Ata-ul-karim, et al. [22] showed that the curve equation of the change of critical nitrogen concentration constructed in rice planting areas of southern China is sensitive to climate differences and rice varieties. In 2010, Ziadi et al. [39] also reported that the $N_c$ dilution curve of winter wheat developed by Justes et al. [24] was different from their research results and proposed that climatic conditions were possible factors causing the difference. Additionally, the effective cumulative temperature in Heilongjiang is 2000 degrees lower than that in the Yangtze River basin, which affects the growth rate of rice. Therefore, the $N_c$ value in this study is low due to various factors, including those mentioned here.

Different rice genotypes have different sensitivities to nitrogen fertilizer. The results from this research show that Wuyoudao4 has a low nitrogen efficiency, while Songjing9 has a high nitrogen efficiency. This study shows that the optimal amount of nitrogen application for Wuyoudao4 is 120 kg·ha$^{-1}$, while that of Songjing9 is 180 kg·ha$^{-1}$.

The critical nitrogen concentration dilution curve model established in this study has clear biological significance and can be used to diagnose the nitrogen nutrition status of rice. This model has certain predictability and accuracy, and the results of this study can be used to accurately diagnose the nitrogen nutrition of rice. This can be helpful for improving accuracy in the management of rice production. Notably, the results of this study are based on field experimental data for only three years; a more extensive study ought to be developed to examine diverse ecological points and further supplement the current data. This would help to achieve an effective unified model to accurately and universally estimate optimal growth. An ideal model could be developed for different types of rice varieties under different environments of nitrogen nutrition for accurate diagnosis and effective crop management.

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