Field Crops Research 167 (2014) 76–85

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

# Determination of critical nitrogen dilution curve based on leaf area index in rice

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ARTICLE INFO

*Article history:*
Received 8 June 2014
Received in revised form 16 July 2014
Accepted 17 July 2014
Available online 13 August 2014

*Keywords:*
Critical nitrogen concentration
Leaf area index
Nitrogen diagnosis
Nitrogen nutrition index
Accumulated nitrogen deficit
Rice.

ABSTRACT

The environmental constraint caused by intensification of rice production in China has raised the need to adjust crop nitrogen (N) fertilizer requirement. Leaf area index (LAI)-based assessment of crop N status can be an effective approach to optimizing N management in rice production. This study was designed to develop a new methodology for establishing the critical nitrogen ($N_c$) dilution curve based on LAI, to compare if a $N_c$ dilution curve based on LAI would allow a better diagnosis of N nutrition status than that on plant dry matter (PDM) basis and to assess its applicability for estimating plant N status in Japonica rice (*Oryza sativa* L) in east China. Three field experiments with varied N rates (0–360 kg N $ha^{-1}$) were conducted using three Japonica rice hybrids, Lingxiangyou-18 (LXY-18), Wuxiangjing-14 (WXJ-14) and Wuyunjing (WYJ) in lower Yangtze River reaches. LAI and plant N concentration (PNC) were determined from active tillering to heading for growth analysis in each experiment. The LAI and PNC ranged from 0.29 to 7.46 and 0.99 to 3.11%, respectively, under the varied experiment conditions. The relationship between N uptake and LAI expansion are strictly proportional and robust across the environments. The $N_c$ dilution curve ($N_c = 3.70\text{LAI}^{-0.35}$) was validated for N-limiting and non-N-limiting growth conditions. The N nutrition index (NNI) and accumulated N deficit ($N_{and}$) ranged from 0.69 to 1.06 and 88.8 to −7.54 kg $ha^{-1}$, respectively, over main growth stages. The values of $\Delta N$ derived from either NNI or $N_{and}$ could be used as references for in-season N dressing management in rice. Our results indicate that $N_c$ dilution curve as a function of LAI efficiently identified the conditions of limiting and non-limiting N nutrition and can be adopted as a novel tool for evaluating plant N status for precision N management in Japonica rice of east China.

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## 1. Introduction

Nitrogen (N) is the most important limiting factor for crop productivity after water deficit and its judicious application has become a burning issue during recent years, especially in the case of rice production in east China. Rice production in China has increased more than three times in the past five decades, rightly attributed to high N fertilizer application rates along with advanced crop management practices (Peng et al., 2009). Despite being a dominant factor in rice production and quality improvement, the excessive use of N fertilizer has reduced N use efficiency and caused severe environmental threats (Jeuffroy et al., 2002). The precise N management has been the most prominent issue these days in the modern agriculture, not just because of economic reasons, but also to curtail the damage caused by excessive N fertilizer to environment. Plant-based diagnostic techniques are required to assess the critical N concentration ($N_c$) in crop plants for optimum growth. The $N_c$ dilution curves describe N nutrition status of a crop which can be used to estimate total N requirements of crops under various field conditions for reliable fertilizer recommendations (Greenwood et al., 1990). These methods were generally based on the N concentration of whole plant dry matter (PDM) or on specific plant parts (e.g. leaves and stem). The existing $N_c$ dilution curves for different crop species including winter wheat (Justes et al., 1994; Yue et al., 2012), corn (Ziadi et al., 2008), spring wheat (Ziadi et al., 2010) and Indica rice (Sheehy et al., 1998) have been established on PDM basis. Similar approach was used by Ata-Ul-Karim et al. (2013) to develop the $N_c$ dilution curve in Japonica rice (using the data set as used

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http://dx.doi.org/10.1016/j.fcr.2014.07.010
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in present study). The $N_c$ dilution curve can be generally described by

$$N_c = aW^{-b} \quad (1)$$

Although PDM-based techniques can offer sufficient knowledge of the limiting and non-limiting factors governing N demand, yet certain limitations restrict its adaptation in modern agricultural practices. It requires destructive and time-consuming procedures for determination of the actual PDM and PNC at different growth stages with representative sampling areas, oven drying and sample grounding. These procedures are out of the expertise of farmers and of the time they could use to obtain the required information (Lemaire et al., 2008). Additionally, it cannot cope with the variation associated with high degree of in-season spatial distribution of crop N status within entire field (Fitzgerald et al., 2010). This approach is also week to offer an insight into correlation of light interception with N nutrition of plant canopies as the investigation of photosynthesis in relation to tissue N content is most meaningful when both variables are expressed in terms of leaf area. Leaf area expansion can be used as an alternative functional approach for assessing N demand because both PDM and leaf area expansion are affected by the same N regulatory process during plant growth (Lemaire et al., 2008). In particular, leaf area expansion under N deficit conditions is limited and causes differential responses in PNC on PDM basis as compared to leaf area index (LAI) basis due to variations in specific leaf weight. The specific leaf weight due to accumulation of DM in the form of starch and cell wall can vary up to two-fold under such conditions and can confuse the relationship between photosynthesis rates and PNC when the latter is expressed in relation to PDM (Koch et al., 1988).

Leaf area expansion may play a major role in N uptake during vegetative growth and previous studies have shown a significantly positive correlation between plant N uptake and LAI under adequate supply of N fertilizer (Plénet and Lemaire, 1999). The relationship between LAI and PDM under N-limiting and non-N-limiting growth conditions was similar which confirms the robustness of this relationship and indicated that isometric growth between leaf area and PDM was not strongly modified by N deficiency (Plénet and Lemaire, 1999). However, it is still uncertain whether PDM or LAI determines plant's demand for N precisely. A strong and undisputed physiological basis for determination of N demand is yet to be established.

Leaf area index could be used as a new approach for determination of the $N_c$ dilution curve in crop (Zhao et al., 2014). LAI is a fundamental variable in agronomic and environmental studies and can be used as a reference tool for addressing various agricultural issues including growth monitoring, yield forecasting and management optimization in crop production. It is helpful to measure canopy light interception and photosynthesis capacity of a crop (Fassnacht et al., 1994). Assimilation of plant photosynthates depends upon leaf area expansion, which are transformed to PDM and are responsible for plant development and maturity (Forde, 2002). During vegetative phase, leaf area expansion is a very important factor to absorb N from the soil and also increases the capacity of the plant to store organic N in Rubisco (Forde, 2002). LAI-based approach can be considered a potential alternative to overcome the problems associated with PDM-based approach for assessment of crop N status, as it can be easily, rapidly and accurately estimated by non-destructive techniques and by remote sensing. Remote-sensing technologies have limited capability to estimate PDM accurately, as the recorded spectral responses are mainly related to the interaction between the sun radiance and plant canopies. Thus, the correlation between PDM and spectral responses or vegetation indices is usually poor under closed canopy for which spectral responses become saturated and lose sensitivity to PDM (Ghasemi et al., 2011). This implies that LAI is an excellent and rapid indicator reflecting the changes in plant N, as compared to the PDM, and can be helpful for in-season management of crop N. Plant N estimation with this technique is eco-friendly and is suitable for multi-scale and multi-temporal research ranging from leaf to landscape and regions (Zheng and Moskal, 2009). Thus, LAI-based diagnostic tools could be more useful for evaluating N status in crop plants.

The present study was intended to establish a new $N_c$ dilution curve based on LAI in Japonica rice, to compare if a $N_c$ dilution curve based on LAI would allow a better diagnosis of N nutrition status than that on PDM basis and to evaluate the acceptability of newly developed $N_c$ curve to estimate the N nutrition status for rice production in east China. The projected results would provide a new methodology for indirect estimation of crop N status and can be used for guiding precision N management during growth period of Japonica rice.

## 2. Materials and methods

### 2.1. Site description

The study area is located in Jiangsu province of east China (32°16′ N, 119°10′ E and 31°56′ N, 118°59′ E), which is an alluvial plain of Yangtze River. The region is one of the major agricultural regions of China and contributes more than 65% of the national rice production in China. The region receives 2176.7 h of sunshine and 1030 mm of rainfall annually, with an average temperature of 15 °C. Two sites were selected for this study, with the same soil type (Ultisoles soil). For site 1 in 2010, soil pH, organic matter, total N, available phosphorous (P) and available potassium (K) were 6.2, 17.5 g $kg^{-1}$, 1.6 g $kg^{-1}$, 43 mg $kg^{-1}$, and 90 mg $kg^{-1}$, respectively, and in 2011 the corresponding soil properties were 6.4, 15.5 g $kg^{-1}$, 1.3 g $kg^{-1}$, 38 mg $kg^{-1}$ and 85 mg $kg^{-1}$, respectively. For site 2 in 2007, soil pH, organic matter, total N, available phosphorous (P) and available potassium (K) were 6.5, 13.5 g $kg^{-1}$, 1.13 g $kg^{-1}$, 45 mg $kg^{-1}$ and 91 mg $kg^{-1}$, respectively.

### 2.2. Experimental design

Three field experiments involving multiple N rates (0–360 kg N $ha^{-1}$) were conducted at these two sites using three contrasting Japonica rice hybrids, Lingxiangyou-18 (LXY-18), Wuxiangjing-14 (WXJ-14) and Wuyunjing (WYJ). The data used to construct the $N_c$ dilution curve based on LAI came from two experiments conducted at site 1 in 2010 and 2011, including five N fertilizer rates, ranging from zero to non-limiting amounts of N. The data for validation of $N_c$ dilution curve mainly came from an independent experiment conducted at site 2 in 2007 with three N fertilizer rates, ranging from N limiting to non-limiting amounts of N.

In each experiment, the treatments of varied N rates were arranged in a randomized complete block design with three replicates. The plot size was 35 $m^2$ with planting density of approximately 22.2 hills per $m^2$. For site 1 in 2010 and 2011, N treatments consisted of five N rates as 0, 80, 160, 240 and 320 kg N $ha^{-1}$, and 0, 90, 180, 270 and 360 kg N $ha^{-1}$, respectively, while for site 2 in 2007, N treatments consisted of three N rates as 110, 220 and 330 kg N $ha^{-1}$. N in all experiments was distributed as 50% at pre-planting, 10% at tillering, 20% at jointing and 20% at booting, with urea as the N source. Phosphorus and potassium fertilizers were incorporated into the soil before transplanting as monocalcium phosphate $Ca(H_2PO_4)_2$ and potassium chloride (KCl) at the rate of 135 kg $ha^{-1}$ ($P_2O_5$) and 190 kg $ha^{-1}$ ($K_2O$), respectively. Rice seedlings were transplanted in experimental fields on June 20 (site

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