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Development of critical nitrogen dilution curve in rice based on leaf dry matter

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ABSTRACT

The critical nitrogen (N_c), defined as the minimum N concentration required for maximum growth, is proposed for diagnosis of the in-season N status in crop plants. It has been established for several crops including rice on whole-plant dry matter (DM) basis but has not been determined for canopy leaf basis. This research was undertaken to develop a new N_c dilution curve based on leaf dry matter (L_{DM}) and to assess its applicability to estimate the level of N nutrition for Japonica rice in east China. Three field experiments were conducted with varied N rates (0–360 kg N ha^{-1}) and three Japonica rice (*Oryza sativa* L.) hybrids, Lingxiangyou-18 (LXY-18), Wuxiangjing-14 (WXJ-14) and Wuyunjing (WYJ) in Jiangsu province of east China. Five hills from each plot were sampled from active tillering to heading for growth analysis and leaf N determination. The N_c dilution curve on leaf N concentration was described by the equation $N_c = 3.76W^{-0.218}$, when L_{DM} ranged from 0.67 to 4.25 t ha^{-1}. However, for $L_{DM} < 0.67$ t ha^{-1}, the constant critical value $N_c = 4.09\% L_{DM}$ was applied. This N_c dilution curve on L_{DM} basis was slightly higher than the curves on plant DM basis in Japonica rice, yet both lower than the reference curve of high yielding Indica rice in tropics. The N nutrition index (NNI) and accumulated N deficit (N_{and}) of leaves ranged from 0.65 to 1.06 and 79.62 to −6.39 kg ha^{-1}, respectively, during main growth stages under varied N rates in 2010 and 2011. The results indicate that the present N_c dilution curve and derived NNI and N_{and} adequately identified the situations of N-limiting and non-N-limiting nutrition in two rice varieties and could be used as reliable indicators of N status during growth of Japonica rice in east China.

1. Introduction

Nitrogen (N) availability is an important factor of crop production, while its deficiency can cause severe reduction in yield and economic returns to growers. N is required in considerable amounts during both canopy establishment and grain quality formation in cereal crops. A large amount of N taken up during active tillering to the end of anthesis is stored in vegetative tissues and then translocated to grain for protein accumulation. However, excessive use of N during last few decades is an emerging threat to environment in many rice production regions. Increased economic costs and environmental concerns associated with N fertilization has made N use efficiency critically important in developing sustainable production systems especially for Japonica rice grown in the lower Reaches of Yangtze River in China, a heavily N fertilized area. Time and rate of N application are two major agronomic management factors affecting N uptake, partitioning and use efficiency (Sinclair and Horie, 1989; Chen et al., 2011). There is an increased interest in identifying when N is most critical for maximum yield. The adjustment of N input to an economically and ecologically compatible level would require quantitative information on the N status of rice plant. An ideal indicator of crop N status should be able to detect deficiencies and excesses of N supply and provide a fast diagnosis to allow dressing correction for efficient fertilizer management during crop growth.

Current tools for detecting N deficiencies in crop plants during growing season include chlorophyll meter readings, remote sensing techniques, soil sampling and destructive plant sampling (N concentration). N concentration can be used on whole-plant DM basis or on specific plant organ (e.g. leaves and stems), such as in concept of critical nitrogen. Plant-based diagnostic techniques are required to assess the N concentration suitable for crop plants,

Abbreviations: L_{DM}, leaf dry matter; DM, dry matter; DAT, days after transplantation; LAI, leaf area index; LSD, least significant difference; LXY-18, Lingxiangyou-18; N_c, critical nitrogen concentration in leaves; N_t, total N concentration in leaf dry matter; N_{na}, actual nitrogen accumulation under different nitrogen rates; N_{and}, accumulated nitrogen deficit; N_{cna}, critical nitrogen accumulation; N_{min}, minimum nitrogen concentration; N_{max}, maximum nitrogen concentration; NNI, nitrogen nutrition index; WXJ-14, Wuxiangjing-14; WYJ, Wuyunjing; YR, Yangtze River Reaches.

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i.e. the minimum N concentration required for maximum growth (Ulrich, 1952). The critical N (N_c) dilution curve for a crop could be determined for indicating N nutrition status of plant, and is represented by a power equation according to Greenwood et al. (1990):

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

where W is the total aerial biomass expressed in t ha^{-1}; N_c is the total N concentration in aerial parts expressed as percentage of dry matter; a and b are positive constants, where a represents the N concentration in the L_{DM} when W = 1 t ha^{-1}, and b is a statistical parameter governing the slope of the relationship.

The N_c dilution curves have been determined on whole-plant DM basis for a number of species, including winter wheat (*Triticum aestivum* L.) (Justes et al., 1994; Yue et al., 2012), potato (*Solanum tuberosum* L.) (Greenwood et al., 1990; Bélanger et al., 2001), winter rapeseed (*Brassica napus* L.) (Colnenne et al., 1998), corn (*Zea mays* L.) (Ziadi et al., 2008), grain sorghum (*Sorghum bicolor* L.) (Van Oosterom et al., 2001), tomato (*Lycopersicon esculentum* Mill.) (Tei et al., 2002), and spring wheat (*T. aestivum* L.) (Ziadi et al., 2010). In rice crop, the similar allometric function based on plant dry matter has been developed by Sheehy et al. (1998) for high yielding Indica rice (*Oryza sativa* L.) in tropics, and by Ata-Ul-Karim et al. (2013) for Japonica rice in east China. Despite being widely accepted, this is not always the most suitable method to determine the weight/N concentration relationship, because dry matter partitioning between different plant organs vary under various abiotic stresses, which ultimately changes the shape of dilution curve (Kage et al., 2002; Vouillot et al., 1998). Although the concept of N_c dilution curve for specific plant organ (e.g. leaves and stem) is similar to that on whole-plant DM basis, yet these corresponding N_c dilution curves remains to be determined on specific plant organ basis in agronomic crops including rice.

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 (Kage et al., 2002; Vouillot et al., 1998). Besides, the leaf is a major photosynthetic organ, and highly responsive to N fertilization (Novoa and Loomis, 1981; Evans, 1989). Thus, it appears that leaf based N_c dilution curve could be more useful for assessing N status in crop plants. Therefore, the current study was aimed first to develop a new N_c dilution curve based on L_{DM}, and then to assess its applicability for estimation of N nutrition level in Japonica rice of east China. The expected results would provide a new approach for diagnosis of tissue N status during growth period of Japonica rice.

2. Materials and methods

2.1. Experimental design

Three field experiments were conducted with varied N rates (0–360 kg N ha^{-1}) in three Japonica rice (*O. sativa* L.) hybrids, Lingxiangyou-18 (LXY-18), Wuxiangjing-14 (WXJ-14) and Wuyunjing (WYJ), in Jiangsu province of east China, as detailed in Table 1. Data used to develop the N_c dilution curve came from two experiments conducted in 2010 and 2011 that included five N fertilizer rates ranging from zero to non-limiting amounts of N. The data for validation of N_c dilution curve came from an independent experiment conducted in 2007 with three levels of N fertilizer, ranging from N limiting to non-limiting amounts of N.

2.2. Plant sampling and tissue N determination

During the period of each experiment, plants for growth analysis were sampled from five hills in each plot at the intervals of 10–12 days from active tillering to heading. The sampling dates for each experiment are presented in Table 1. The plants were severed at ground level on each sampling date. Fresh plants were separated into different leaves (1st leaf from top, 2nd leaf from top, 3rd leaf from top and rest of leaves), and stem (culm plus sheath).

Plant samples were oven-dried at 80 °C for 48 h, and weighed for L_{DM} (t ha^{-1}). Then, the leaf samples were ground to powder to pass through a 1-mm sieve, and stored in plastic bags at room temperature until further analysis. Total N concentration in leaf samples was determined by using micro-Kjeldahl method (Bremner and Mulvancy, 1982). Leaf N accumulation (LNA) was obtained as summed products of the L_{DM} of different leaves by the N contents in the corresponding leaf on DM basis.

$$\mathrm{LNA} = \frac{L_{1DM}L_{1N} + L_{2DM}L_{2N} + L_{3DM}L_{3N} + L_{RDM}L_{RN}}{100} \quad (2)$$

where L_{DM} is dry matter (t ha^{-1}) of different leaves , and L_N is N concentration of corresponding leaf, respectively; L_R is rest of leaves other than the top 3 leaves.

Then, the leaf nitrogen concentration (LNC, N_t) of whole leaf canopy was calculated as LNA divided by total L_{DM} .

$$\mathrm{LNC} = \frac{\mathrm{LNA}}{L_{DM}} \quad (3)$$

2.3. Data analysis

Critical nitrogen (N_c) values were determined by the methodology described by Justes et al. (1994). Data from each sampling date were divided into two groups, namely (i) N-limiting, where increasing N supply resulted in a significant response in L_{DM} and N concentration, and (ii) non-N-limiting, where additional N supply did not lead to further increase of L_{DM} but only of N concentration. These data points were used either for construction of the N_c dilution curve, or to validate it. The differences among treatment means were measured by using the least significant difference (LSD) test at 90% level of significance, instead of classically using 95% in order to reduce the occurrence of Type II errors which could be higher in such field experiments. For each sampling date and year, this division was achieved when data of L_{DM} and corresponding N concentrations from different N rates were subjected to analysis of variance (ANOVA) using GLM procedures in SPSS-16 software package (SPSS Inc., Chicago, IL, USA). In a second step, regression lines were calculated separately for each group of data points using Microsoft Excel (Microsoft Cooperation, Redmond, WA, USA).

The N concentration at the intersection point of these two regression lines constituted the desired critical value. An allometric function was used to determine the relationship between N_c and dry matter of leaves for these data points. The critical curve was validated first by using the data points not retained for establishing the parameters of the allometric function and then with independent data set from 2007.

Further, the N nutrition index (NNI) of rice leaves at each sampling date was determined as the total N concentration of leaves (N_t) divided by N_c, according to previous reports on potato (Bélanger et al., 2001) and corn (Ziadi et al., 2008), as Eq. (4).

$$\mathrm{NNI} = \frac{N_t}{N_c} \quad (4)$$

The difference value of NNI (ΔNNI) between different N treatments was calculated as Eq. (5), according to the method proposed by Ata-Ul-Karim et al. (2013).

$$\Delta\mathrm{NNI} = \mathrm{NNI}i - \mathrm{NNIck}, \quad (i = 1, 2, 3, 4, 5) \quad (5)$$