



# Determining of a critical dilution curve for plant nitrogen concentration in winter barley

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## ABSTRACT

Accurate forecasting of nitrogen (N) required for plant growth can serve as a guide to improving agricultural practices and regulations. The objectives of this study were to: (i) establish a critical N ( $N_c$ ) dilution curve for winter barley based on the shoot dry matter (DM), (ii) compare this curve with other  $N_c$  dilution curves of different species, and (iii) assess the plausibility of using this curve to estimate the N status of winter barley. Four field experiments were conducted with different N application rates. Twenty plants from each plot were sampled from the Feekes 3 to Feekes 10.51 stage for growth analysis. The relationship between  $N_c$  and shoot DM could be described by a negative power equation,  $N_c = 4.76DM^{-0.39}$ , when shoot DM values were in the range of 1.79–13.69 t ha<sup>-1</sup>. For DM values <1.79 t ha<sup>-1</sup>, however, the constant value  $N_c = 3.77\%$  was used. The N nutrition index (NNI) ranged from 0.39 to 1.39 from Feekes 3 to Feekes 10.51 under different N application rates from 2005 to 2007. The  $N_c$  dilution curve identified limiting and non-limiting N status of winter barley and could be used as a reliable indicator of in-season N stress of winter barley grown.

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## 1. Introduction

Winter barley is the major cereal crop in the world, as well as in China. The grain of winter barley can be used for malting, feedstock and consumption. The rate of N fertilizer application is important for winter barley since N can affect growth stage, grain yield and quality (Poehlman, 1985). More N fertilizer can increase the yield and protein of winter barley, but the thousand kernel weight and quality of winter barley decrease (Grant et al., 1991). In the winter barley production, about 150–225 kg ha<sup>-1</sup> N has been applied annually in China, with 70% as basal fertilizer (Xu et al., 2010). It has been reported that losses may reach 50% of the amount of fertilizer applied because of the poor synchrony between N supply and crop demand (Brye et al., 2003; Guarda et al., 2004), particularly early in development when the root mass of the cereal crops is low (Raun and Johnson, 1999; Fageria and Baligar, 2005), which can induce the decrease of N use efficiency. N use efficiency of the cereal crops is estimated as 40 and 29% under the developed countries and undeveloped countries (Hodge et al., 2000; Zhu and Wen, 1992). Further, high N-fertilizer consumption is also environmentally damaging, with excess N lost by atmospheric emissions and water or soil

pollution via leaching (Kaye and Hart, 1997; Galloway et al., 2008). Therefore, the optimization of N requirements at different developmental stages of winter barley is a core issue of research for N use efficiency and environmental protection.

Plant-based analytical techniques of N deficiency can be a priori diagnosis to determine the necessity of applying additional N fertilizer. Current tools for detecting N deficiencies in crop plants during growing season include chlorophyll meter readings, remote sensing techniques (Fox et al., 1994; Hansen and Schjoerring, 2003), but these techniques are easily affected by different environments and are limited use for detecting excess N uptake (Dwyer et al., 1995; Feng et al., 2008). Therefore, an alternative technique is to use the concept of  $N_c$  dilution concentration, which represent the minimum N concentration required for maximum crop growth (Ulrich, 1952). The concept of a  $N_c$  dilution curve based on plant N concentration (PNC) was developed by Lemaire and Salette (1984) for tall fescue and is represented by an allometric function:

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

where DM is the shoot DM expressed in t ha<sup>-1</sup>,  $N_c$  is the critical N concentration in shoots expressed in % DM, and  $a_c$  and  $b$  are estimated parameters. The parameter  $a_c$  represents the  $N_c$  concentration in the shoot DM for 1 t ha<sup>-1</sup>, and the parameter  $b$  represents the coefficient of dilution describing the relationship between PNC and shoot DM. Greenwood et al. (1990) proposed two

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**Table 1**

Basic information about four field experiments conducted in Huaian and Anyang.

	Season	Soil characteristics (20 cm)	Cultivar	N rate (kg N ha <sup>-1</sup> )	Sampling stage
Experiment 1 Huaian	2005/2006	Type: sandy soil	Supi6 (SP6)	0 (N0)	Feekes 3
		Organic matter: 14.26 g kg <sup>-1</sup>	Yangpi4 (YP4)	75 (N1)	Feekes 7
		Total N: 1.2 g kg <sup>-1</sup>		150 (N2)	Feekes 10
		Available P: 64 mg kg <sup>-1</sup>		225 (N3)	Feekes 10.51
Experiment 2 Huaian	2006/2007	Available K: 80.5 mg kg <sup>-1</sup>		300 (N4)	
		Type: sandy soil	Supi6 (SP6)	0 (N0)	Feekes 3
		Organic matter: 11.43 g kg <sup>-1</sup>	Yangpi4 (YP4)	75 (N1)	Feekes 7
		Total N: 1.1 g kg <sup>-1</sup>		150 (N2)	Feekes 10
Experiment 3 Anyang	2007/2008	Available P: 13.34 mg kg <sup>-1</sup>		225 (N3)	Feekes 10.51
		Available K: 121 mg kg <sup>-1</sup>		300 (N4)	
		Type: clay soil	Yangnongpi8	0 (N0)	Feekes 3
		Organic matter: 17.9 g kg <sup>-1</sup>	(YNP8)	90 (N1)	Feekes 7
Experiment 4 Anyang	2007/2008	Total N: 1.3 g kg <sup>-1</sup>		180 (N2)	Feekes 10
		Available P: 43 mg kg <sup>-1</sup>		270 (N3)	Feekes 10.51
		Available K: 92 mg kg <sup>-1</sup>			
		Type: clay soil	Supi2 (SP2)	0 (N0)	Feekes 3
		Organic matter: 12.5 g kg <sup>-1</sup>	Dan2 (D2)	90 (N1)	Feekes 7
		Total N: 1.2 g kg <sup>-1</sup>		180 (N2)	Feekes 10
		Available P: 63 mg kg <sup>-1</sup>		270 (N3)	Feekes 10.51
		Available K: 102 mg kg <sup>-1</sup>			

general relationships between  $N_c$  and shoot DM: one for  $C_3$  species ( $a_c = 5.7$  and  $b = 0.5$ ) and one for  $C_4$  species ( $a_c = 4.1$  and  $b = 0.5$ ). These relationships showed that  $C_4$  species accumulated 25% more DM than  $C_3$  species with same amount of N taken up, the declining slope of PNC was the same in all plants irrespective of their carbon metabolism, and depends mainly on light interception (Le Bot et al., 1998). Both of these equations have been useful for N diagnoses, but their accuracy is limited due to differences in histological, morphological, and ecophysiological characteristics among species, so every species should have its own  $N_c$  dilution curve (Lemaire and Gastal, 1997). Further, the  $N_c$  takes a constant value due to the small decline of  $N_c$  with increasing shoot DM ( $b = 0.12$ – $0.15$ , Lemaire et al., 2007). The  $N_c$  dilution curves have been determined for various crops, such as tomato (Tei et al., 2002), winter canola (Colnenne et al., 1998), maize (Plénet and Lemaire (2000)), rice (Sheehy et al., 1998) and winter wheat (Justes et al., 1994). However, a  $N_c$  dilution curve has never been determined for winter barley. Although barley and wheat are of the same family, they differ morphologically and ecophysiological characteristics, and it is unclear whether or not their critical N curves are similar.

In the present study, our objectives were to determine and validate the  $N_c$  dilution curve of winter barley, to compare this  $N_c$  dilution curve with the existing  $N_c$  dilution curves of other crops, and to explore the plausibility of estimating the N status of winter barley.

## 2. Materials and methods

### 2.1. Experimental design

Data were obtained from four field experiments in Huaian (32°43' N, and 119°36' E) and Anyang (36°05' N, and 114°21' E) involving various N rates (0–300 kg ha<sup>-1</sup>) and winter barley cultivars (SP6, YP4, YNP8, SP2 and D2) at different sites and in different years, as summarized in Table 1. All the experiments consisted of a completely randomized block design with three replicates. The winter barley was planted in early-November and harvested in mid-May the following year. N fertilizer was applied before the sowing stage (70%) and at the jointing stage (30%). All experiments received appropriate amounts of triple superphosphate (0–150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium chloride (0–120 kg K<sub>2</sub>O ha<sup>-1</sup>) before planting. Further crop management procedures followed common agricultural practices to ensure maximum potential productivity, i.e., no factor other than N was limiting.

The results of Experiments (Exp)1 and 2 were used to construct a  $N_c$  dilution curve. The results from Exp 3 and 4 were used to test the accuracy of this curve.

### 2.2. Sampling and measurement

We collected 20 plants per plot (24 m<sup>2</sup>) at different Feekes stages for determination of shoot DM and PNC. Shoot DM was obtained by a forced-draft oven drying at 80 °C to constant weight, followed by analytical balance weighing. Dried leaves were ground in a sample mill, passed through a 1-mm sieve, and stored in plastic bags for chemical analysis. PNC was determined by the micro-Kjeldahl method (Bremner and Mulvancy, 1982).

### 2.3. Data analysis

Determination of a critical N curve requires identifying critical data points at which N neither limits nor enhances plant growth. Data from experiments conducted in 2005–2007 are shown in Table 1. An N-limiting growth treatment is defined as a treatment in which any additional N application would lead to a significant increase in shoot DM. A non-N-limiting growth treatment was similarly defined as an N application rate that would not lead to an increase in shoot DM but would result in a significant increase in PNC (Justes et al., 1994). The shoot DM and PNC under different N treatments were compared by an ANOVA (SPSS) at the 5% probability level. For one sampling date, a critical point was defined as follows: (i) each N treatment was characterized by the shoot DM and PNC; (ii) data from the N-limiting growth conditions were fitted by a simple linear regression (the oblique line); (iii) data from the non-N-limiting growth conditions were used to calculate the maximum shoot DM as the average of the observed data (the vertical line); (iv) the theoretical critical point was determined as the ordinate of the intersection point of the oblique and vertical lines; and (v) an allometric equation was fitted to these critical points to determine the equation of the  $N_c$  dilution curve.

Data points were selected from only non-N-limiting treatments (N4 treatments in 2005–2007) for determination of the maximum N curve ( $N_{max}$ ); the data points from N-limiting treatments (N0 treatments in 2005–2007) were used to construct the minimum N curve ( $N_{min}$ ).

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