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Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings

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ABSTRACT

Various indices established on a portable SPAD-502 meter can serve as indicators of the nitrogen status of a plant. We compared di-positional SPAD readings and indices with several reliable nitrogen indicators during vegetative growth stage of rice (*Oryza sativa* L.) and developed a prediction model for diagnosing nitrogen status. Three field experiments were conducted in Jiangsu province of east China during 2013 and 2014. Different nitrogen application rates were used to generate contrasting conditions of nitrogen availability in three Japonica, Wuyunjing 19, Yongyou 8, and Wuyunjing 24, and one Indica rice hybrid, Yliangyou 1. The SPAD values of the uppermost four fully expanded leaves were measured from tillering to heading stages, and these values were further used to calculate the normalized SPAD index (NSI), relative SPAD index (RSI), difference SPAD index (DSI), relative difference SPAD index (RDSI), and normalized difference SPAD index (NDSI). Five hills from each plot were simultaneously sampled, and four nitrogen indicators, including leaf nitrogen concentration (LNC), plant nitrogen concentration (PNC), plant nitrogen accumulation (NA), and nitrogen nutrition index (NNI), were measured. The results of linear correlations among the SPAD and nitrogen indicators indicated that NSI of the fourth fully expanded leaf from top (NSI4) was the most reliable and generally applicable SPAD indicator. In total, 24 potential single-stage and duration models were established, taking into consideration the fact that duration models covering the period from stem elongation to booting stages are more robust, two duration diagnostic models (Model 1: $NA = 0.0279e^{8.6957NSI4}$, $R^2 = 0.730^{**}$, $n = 45$; Model 2: $NNI = 0.0163e^{4.13NSI4}$, $R^2 = 0.767^{**}$, $n = 45$, NSI4 = 0.80–1.00) were developed and calibrated. Both models can provide accurate and appropriate N diagnosis from the stem elongation to booting growth stages for rice production.

Abbreviations: BT, booting; DAS, days after sowing (days); DAT, days after transplanting (days); DM, dry matter (kg ha^{-1}); DSI, difference SPAD index; H-43, Hang43; HD, heading; L1, SPAD reading of the first fully expanded leaf from top; L2, SPAD reading of the second fully expanded leaf from top; L3, SPAD reading of the third fully expanded leaf from top; L4, SPAD reading of the fourth fully expanded leaf from top; LDM, leaf dry matter (kg ha^{-1}); LFT1, first fully expanded leaf from top; LFT2, second fully expanded leaf from top; LFT3, third fully expanded leaf from top; LFT4, fourth fully expanded leaf from top; LNC, leaf nitrogen concentration; N, nitrogen; NA, nitrogen accumulation (kg ha^{-1}); NDSI, normalized difference SPAD index; NNI, nitrogen nutrition index; NSI, normalized SPAD index; NSI1, normalized SPAD index of the first fully expanded leaf from top; NSI2, normalized SPAD index of the second fully expanded leaf from top; NSI3, normalized SPAD index of the third fully expanded leaf from top; NSI4, normalized SPAD index of the fourth fully expanded leaf from top; PD, panicle differentiation; PDM, plant dry matter (kg ha^{-1}); PI, panicle initiation; PNC, plant nitrogen concentration; RDSI, relative difference SPAD index; RSI, relative SPAD index; SE, stem elongation; TI, tillering; WYJ-19, Wuyunjing19; WYJ-24, Wuyunjing24; XS-63, Xiushui63; YLY-1, Yliangyou1; YY-8, Yongyou8.

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1. Introduction

After water, nitrogen (N) is arguably the most important limiting factor for crop production. Optimal N management is crucial for high-efficiency crop production and sustainable agriculture. In rice (*Oryza sativa* L.), N deficiency leads to low shoot biomass and reduced yield, while excessive N causes disease and a range of environmental issues (Ghosh and Bhat, 1998; Xie et al., 2007). China's national average rate of N usage in rice cultivation (193 kg/ha) is ~90% higher than the global average (Heffer, 2009). The average rate of N fertilizer application in Jiangsu province of east China reached 387 kg ha^{-1} during 2004–2008 (Chen et al., 2011). The high rates of N fertilizer input and improper timing of application have led to low efficiency in agronomic N use in the country. One of the reasons for this improper use of N in Chinese agriculture is the lack of practical diagnostic tools for N in crop production.

The two principal approaches for satisfying crop N demand are the use of diagnostic methods for both plant-based and soil-based N (Balasubramanian et al., 1998). In the past, leaf N concentration (LNC) has been used to monitor the N status of rice crops (Peng et al., 1993; Tian et al., 2011). However, this method, which uses leaf sampling and laboratorial analysis, is destructive, time-consuming, and expensive. Indirect measurement of LNC provides a simple, rapid, and nondestructive way to estimate the N content of rice leaves. Among the various indirect methods, the most widely adopted is detection based on the chlorophyll meter. Generally, the first or second uppermost fully expanded leaf is sampled to determine crop N nutrition (Peng et al., 1996). However, recent studies have shown that there are large differences in the sensitivity of the response to increased N rates between the upper and lower leaves in rice; the lower leaves appear to be more sensitive to N levels than the upper leaves, and the former could be more suitable as test samples for N status diagnosis (Wang et al., 2006). Other studies have found that the chlorophyll meter reading can be affected by many factors, such as irradiance, leaf thickness, growth stage, and genotype (Schepers et al., 1992; Blackmer and Schepers, 1995; Samborski et al., 2009). To compensate the factors other than N that affect the chlorophyll content, the normalized SPAD index (NSI) was developed, which is calculated by dividing the tested area SPAD reading under various N rates by the value of the fully fertilized reference plot (Blackmer and Schepers, 1995). In addition to the NSI, four additional SPAD indices, the relative SPAD index (RSI), difference SPAD index (DSI), relative difference SPAD index (RDSI), and normalized difference SPAD index (NDSI), have also been studied (Lin et al., 2010). Despite the use of so many SPAD indicators in N diagnostics research, studies have focused on the effect of just one indicator, and there has been no comprehensive comparison of the range of indicators available.

The chlorophyll meter reading is dimensionless, and it is only useful for diagnosing the N status of crops when correlated with reliable N indicators. There are four existing N indicators for crop N management: total plant N concentration (PNC), LNC, N nutrition index (NNI), and plant N accumulation (NA). The traditional plant N index is PNC, which has been studied extensively in numerous crops (Wang et al., 2006). For LNC, many studies have found a close correlation between chlorophyll content and leaf N content measurements (Piekkielek and Fox, 1992; Matsunaka et al., 1997; Errecart et al., 2012). Lemaire et al. (1997) reported that the N concentration of the uppermost illuminated leaves of the canopy in maize remained generally constant under conditions of stable N nutrition status, even when PNC declined as crop biomass increased. Hence, LNC is considered to be a more reliable N indicator, especially for the SPAD meter. The NNI is determined by dividing the actual PNC by a critical N concentration (Nc) (Lemaire and Gastal, 2009). Numerous studies have assessed the relationships among SPAD indicators and NNI in various crops, such as tall fescue (Errecart et al., 2012), durum wheat (Debaeke et al., 2006), rice (Yang et al., 2014), and corn (Ziadi et al., 2008). NA represents the overall growth status of a crop, providing information on both shoot biomass and N concentration. Hence, NA is a useful indicator for crop N diagnosis (Xue et al., 2004). For these four potentially useful N indicators, previous studies gave attempted comparisons between only a single N indicator and SPAD readings, and further research that examines multiple SPAD and N indicators is needed.

In the present study, we comprehensively validated the common linear relationships among SPAD indicators and N indicators to identify the optimal indicators for N diagnosis based on the SPAD meter; established robust N diagnostic models based on the optimal indicators, and assessed their ranges of application. These diagnostic models were calibrated and evaluated for their performance with an independent experimental dataset. The projected results would provide a new approach for indirect estimation of crop N status and can be used for judicious N management during the growth period of rice.

2. Materials and methods

2.1. Experimental design

Three field experiments were conducted with different N application rates (0–375 kg N ha^{-1}) in three Japonica, Wuyunjing-19 (WYJ-19), Yongyou-8 (YY-8), Wuyunjing-24 (WYJ-24), and one Indica rice (*O. sativa* L.) hybrid, Yliangyou-1 (YLY-1), in Jiangsu province of east China, as detailed in Table 1. A randomized complete block design with three replicates was used in all of the experiments; the hill spacing was 0.25 m × 0.15 m with two seedlings per hill in a 5 m × 6 m plot. The banks between the individual plots were covered with plastic film to prevent fertilizer penetration across the treatments. The N fertilizer used was urea with an N content of 46%. The distribution of total N at different growth stages was 50% before transplanting, 20% at tillering, and 30% at booting. Phosphorus and potassium fertilizers were added to the soil before transplanting as monocalcium phosphate $Ca(H_2PO_4)^2$ and potassium chloride (KCl) at rates of 135 kg ha^{-1} (P_2O_5) and 190 kg ha^{-1} (K_2O).

2.2. Plant sampling and N determination

Five hills from each plot were sampled for growth analysis at different growth stages during vegetative growth period from each experiment,. Rice plants were manually uprooted, and cut at ground level for determination of N concentration. Fresh plants were separated into green leaf blade (leaf) and culm plus sheath (stem), heated for 30 min at 105 °C to halt metabolic processes, and dried at 80 °C in a forced-draft oven until they reached a constant weight. Plant dry matter (PDM) was determined, and the samples were ground before passing them through a 1 mm sieve in a Wiley mill. Then the samples were stored in plastic bags at room temperature until further chemical analysis. Samples of 0.2 g dried and ground biomass were digested using a mixture of H_2O_2 and H_2SO_4, and the N content was determined using a continuous-flow autoanalyzer (BRAN + LUEBBE AA3; Germany). Grain yield was determined for a 2 m^2 area in each plot and adjusted to the moisture content of 14%.

2.3. SPAD measurements

Readings were taken with an SPAD-502 (Minolta Camera Co., Osaka, Japan) from the four uppermost fully expanded leaves. The first, second, third, and fourth fully expanded leaves from the top of the plant were named LFT1, LFT2, LFT3, and LFT4, respectively. Ten randomly selected plants from each field plot were measured, and three SPAD values per leaf, including one value around the midpoint