SPAD Values and Nitrogen Nutrition Index for the Evaluation of Rice Nitrogen Status

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Abstract: Plant-based diagnosis is one of the most important methods to determine nitrogen (N) content of crops. Our objective was to establish the relationship between soil-plant analysis development (SPAD) values and N nutrition index (NNI) during the three developmental stages of rice and apply the SPAD meter as diagnostic tools for predicting grain yield response to N fertilization. We determined the SPAD values of four uppermost fully expanded leaves of two rice cultivars at six N fertilization levels at three growth stages and examined the relationship between SPAD values and NNI. The critical N concentration (Nc) was 5.31 W^{0.5} in Xiushui63, and 5.38 W^{0.49} in Hang43, where W is the total shoot biomass. The correlation between SPAD value and NNI varied with the leaf position, developmental stage, and variety. The lower leaf appeared to be more sensitive to the N level than the upper leaf in the response of biomass, and could be more suitable as a test sample for N status diagnosis, especially in the booting and heading stage. The dependence of grain yield on SPAD values of the fourth fully expanded leaf (L4) was significant at booting stage (R^2_L4 = 0.82** in 2011, R^2_L4 = 0.72** in 2012). Ratio of SPAD values of L4 to that in the N-saturated plot (RSPAD) (R^2_L4 = 0.92** in 2011, R^2_L4 = 0.77** in 2012) and NNI (R^2 = 0.96** in 2011, R^2 = 0.86** in 2012) at booting stage demonstrated a closer relationship with grain yield.

Key words: Grain yield, Leaf position, Nitrogen nutrition index, Rice, SPAD values.

Nitrogen (N) is the most important limiting factor, after water deficit, for biomass production in natural ecosystems. In paddy field systems, N fertilization can provide plants a sufficient N supply to achieve the potential grain yield under the vested meteorological conditions. Inadequate N application leads to a decrease in leaf area (Fernandez et al., 1996), chlorophyll contents, leaf photosynthesis, biomass production (Zha and Oosterhuis, 2000), and the loss of yields and qualities. Farmers often apply larger quantities of N fertilizers than strictly required for achieving maximum yield, to ensure potential yield every year. However, excessive use of N fertilizer causes lodging and disease and results in increased input costs and net yield reduction. The nitrate pollution of surface and ground water also occurs when N fertilization exceeds crop requirements (Mary, 1997). Fertilizer N is commonly applied in excess of the requirement to achieve high yields of rice in Southeast China. The annual application rate of synthetic N for conventional agricultural practices in Southeast China (Taihu region) ranges from 550 to 600 kg N ha^{-1} for typical double-cropping systems (Ju et al., 2009). Fertilizer N use efficiency for rice production in Southeast region of China is lower than in other regions for rice production (Wang et al., 2001; Peng et al., 2002). For example, when N fertilizer was applied between 225 and 315 kg ha^{-1} in paddy field, only 28% – 33% of N fertilizer was absorbed by crops in Taihu region (Yan, 2011). These are partly due to the lack of effective diagnostic tools. Therefore, it is important to develop effective diagnosis of rice N status for sustainable management of rice production.

The soil-plant analysis development (SPAD) meter is a low-cost, rapid, simple, and non-destructive apparatus for diagnosis of crop N nutrition that has facilitated research in plant physiological ecology during the past few decades. It is widely used to monitor leaf N status of many crops, including cotton (Wu et al., 1998), durum wheat (Debaeke et al., 2006), maize (Singh et al., 2011) and irrigated rice (Peng et al., 1996; Huang et al., 2008). However, the successful use of SPAD meters can be affected by many factors such as variety, year, growth stage, leaf thickness, leaf positions and the measurement point on the leaf. (Hoel and Solhaug, 1998; Thiyagarajan, 2000; Peng et al., 2006; Esfahani et al., 2008; Huang et al., 2008; Li et al., 2011). To reduce the influence of the above factors, researchers adopted many effective measures. For
example, Peng et al. (1993, 1995) and Esfahani et al. (2008) suggested that the correlation coefficient between SPAD values and leaf N concentration based on per unit area should be higher than that based on dry weight. Li et al. (2009) confirmed this conclusion by investigating leaf thickness. Varvel et al. (1997) and Hussain et al. (2000) used SPAD Sufficiency Index to overcome the influence of varieties, developmental stages and locations on SPAD measurements. The relative SPAD values (RSPAD values) are obtained by dividing the values in the test area by that in an N-saturated plot that has received a high N rate. Wang et al. (2006) and Lin et al. (2010) utilized the difference, or ratio of SPAD values between different leaf positions to predict rice N status for eliminating the influence of genotypes and developmental stages. Hawkins et al. (2007) used RSPAD values to determine N application rates for corn. Coefficients of determination ranging from 0.57 to 0.99 have been reported between relative grain yield (ratio of grain yield to the highest yield) and RSPAD values in corn (Piekielek et al., 1995; Waskom et al., 1996; Scharf et al., 2006).

The N nutrition index (NNI) is the reference plant-based indicator of the N nutrition status of crops. It is defined as the ratio of actual shoot biomass N concentration to critical N concentration (Nc), which is defined as the minimum plant N concentration allowing maximum shoot biomass (Ulrich, 1952). The concept of a critical N curve based on the N concentration in whole plants was first developed by Lemaire and Salette (1984) for tall fescue and has been successfully applied to wheat (Justes et al., 1994), maize (Plénet and Lemaire, 2000), sorghum (Plénet and Cruz, 1997), rice (Sheehy et al., 1998), winter canola (Colnenne et al., 1998), pea (Ney et al., 1997) and tomato (Tei et al., 2002). Nc is represented by an allometric function:

\[ N_c = a_c W^{-b} \]  

where W is the total shoot biomass expressed by Mg ha\(^{-1}\) dry matter, Nc is the shoot N concentration expressed by g kg\(^{-1}\) dry matter, and a_c and b are estimated parameters. The parameter a_c represents the N concentration in the shoot biomass of 1 Mg ha\(^{-1}\) dry matter and the parameter b represents the coefficient of dilution, which describes the relationship of decreasing N concentration with increasing shoot biomass. However, variation in the critical N curve between and within species (Justes et al., 1994; Bélanger et al., 2001), and between experimental sites (Greenwood et al., 1990) has been reported. A validation of these parameters for the pedo-climatic conditions and rice hybrids of Zhejiang, China is therefore required.

The non-linear relationship between SPAD values and NNI has been proved in fescue (Errecart et al., 2012), corn (Ziadi et al., 2008) and wheat (Debaeke et al., 2006). Debaeke et al. (2006) also found that the non-linear relationship between RSPAD values and NNI was not significantly affected by years, genotypes, and developmental stages. This method of relating SPAD or RSPAD values to NNI and grain yield has not been extensively applied to rice. The correlation of NNI with SPAD and RSPAD values in different leaf position of the uppermost canopy with NNI, and grain yield remains unclear in rice plants.

Our main objectives were: (i) to validate the parameters of the critical N curve of Sheehy et al. (1998) for rice hybrids in different years and to assess the plausibility of using this critical N curve to estimate the level of N nutrition in rice, (ii) to establish the correlation of NNI with SPAD and RSPAD values, and RSPAD values in different leaf positions at different developmental stages, and (iii) to compare the two methods as diagnostic tools for predicting grain yield response to N fertilization.

Materials and Methods

1. Experimental design

Two duplicated experiments were conducted at the experimental farm of Hangzhou Academy of Agriculture, Hangzhou, Zhejiang province, China (30°15’N, long 120°7’E) during the growing seasons of 2011 and 2012. The soil type was a clay loam with pH 5.87, organic matter of 35.50 g kg\(^{-1}\) and total N of 2.75 g kg\(^{-1}\) at 0 – 30 cm depth.

Rice cultivars “Xiushui63” (2011) and “Hang43” (2012) were planted and treated with six N levels in a randomized block design with 3 replications. Seedlings with 5 – 6 fully expanded leaves were transplanted on 25 June 2011 and 26 June 2012. Hill spacing was 0.23 m × 0.13 m with 2 seedlings per hill in the 3 m × 6 m plot. Plants received 0, 75, 150, 225, 300, and 375 kg N ha\(^{-1}\) respectively as urea. Each rate of N was applied 3 times according to rice developmental stages: tillering (8 days after transplanting (DAT), 20%), booting (28 DAT, 50%), and heading (62 DAT, 30%). Superphosphate (225 kg ha\(^{-1}\)) and potassium chloride (75 kg ha\(^{-1}\)) were incorporated into every plot at the transplanting day, and additional 75 kg ha\(^{-1}\) potassium chloride was top-dressed on 40 DAT. The banks between the individual plots were covered with plastic film to prevent fertilizer penetration across the treatments.

Crop management followed the standard cultural practices. The experimental field was kept flooded at the depth of 5 to 10 cm from transplanting until 10 days before maturity. Insects and diseases were intensively controlled by chemicals to avoid biomass and grain yield loss. Herbicide was used to control weeds.

2. Plant measurements

Shoot biomass was measured about every three weeks in

\[ \text{Shoot biomass} = \text{Plant production} \times (1 - \text{N loss}) \]
2011 and 2012 using a 1 m² area in each plot. Whole plants were cut at ground level using pruning-scissors. For dry matter determination and laboratory analyses, all plants in the 1 m² area were mechanically shredded and a subsample of approximately 500 g was collected. The subsamples were heated for 30 min at 105°C, and dried at 75°C in a forced-draft oven for 4 days until they reached a constant weight. The dried samples were milled to pass 1 mm screen, and then stored in plastic bags at room temperature before laboratory analyses. Before the determination of N concentration, samples of 0.1 g dried matter was ground into powder, pelleted and wrapped in tin foil which was N free. The N concentration was measured on a rapid N cube (Elementar, Germany), called Dumas combustion method (Jung et al., 2003), and which was expressed in N (%). Grain yield was determined for a 10 m² area in each plot and adjusted to the standard moisture content of 14%.

A SPAD meter (SPAD-502, Minolta Camera Co., Osaka, Japan) was used to take SPAD values from the 4 uppermost fully expanded leaves on each plant at an interval of approximately 7 days. The first, second, third and fourth fully expanded leaves from the top of plant were named L1, L2, L3 and L4, respectively. A total of 10 plants were measured in every plot, and 3 SPAD values per leaf, including one value around the midpoint of leaf blade and 2 values at 3 cm apart from the midpoint were averaged as the mean SPAD value of the leaf (Peng et al., 1993).

### 3. Data Analysis

Data for each sampling date and year were subjected to analyses of variance using SPSS18.0 (Chicago, IL, USA)
and the least significant difference (LSD) test was used to assess differences between treatment means. The NNI of the crop at each sampling date was determined by dividing the N concentration of the shoot biomass by \( N_c \) (Sheehy et al., 1998). \( N_c \), the minimum N concentration required to achieve maximum shoot growth, was determined as a function of shoot biomass as proposed for rice by Sheehy et al. (1998; \( N_c = 5.20 \times W^{-0.52} \) where \( W \) is the total shoot biomass expressed by Mg ha\(^{-1}\) dry matter) and validated for Zhejiang, China. \( N_c \) dilution curve was obtained according to method of Justes et al. (1994). The RSPAD value at each plot was determined by dividing the SPAD value in a given N treatment by the value in the largest dry matter treatment; 225 kg ha\(^{-1}\) N (N4) in 2011 and 300kg ha\(^{-1}\) N (N5) in 2012. Relative grain yield at each plot was computed as the ratio of grain yield for a given N level with the highest grain yield among all N levels (Ziadi et al., 2007). Simple and multiple regression analysis were performed among NNI, SPAD values, relative grain yields and RSPAD values using SPSS 18.0 (Chicago, IL, USA). Intercepts and slopes of regression curve at different developmental stages were compared using the model procedures of SPSS 18.0 (Chicago, IL, USA), Origin8.0 and Excel 2010.

Results and Discussion

1. Changes in SPAD values with time and N fertilization levels

The SPAD values of the two varieties showed a similar change throughout the growing season when N was not
applied (N1) in both years. Both varieties responded to N application quickly by increasing SPAD values. Hang43 had a higher SPAD value than Xiushui63 at the same N rate (Figs. 1 and 2). This difference was mainly due to the higher specific leaf weight of Hang43 than that of Xiushui63 (Huang et al., 2006; Li et al., 2011). SPAD values generally increased to a maximum and then gradually decreased in N application plots (N2-N6) during the growing season. Similar seasonal changes in SPAD values have been reported previously in rice (Jiang et al., 2012).

At the early tillering stage (0–30 DAT), the SPAD values at identical leaf positions were generally not significantly affected by N rates, including the highest N rate, which had similar SPAD values (Figs. 1 and 2), because N restriction on plant growth was not severe at this stage. Peng et al. (1996) suggested that the lack of N restriction at an early tillering stage of rice was probably due to large amount of residual soil N at planting. Because 50% of N had a higher SPAD value than Xiushui63 at the same N rate (Figs. 1 and 2), because N restriction was applied (N1) in both years. Both varieties responded to N application quickly by increasing SPAD values. Hang43 had a higher SPAD value than Xiushui63 at the same N rate (Figs. 1 and 2). This difference was mainly due to the higher specific leaf weight of Hang43 than that of Xiushui63 (Huang et al., 2006; Li et al., 2011). SPAD values generally increased to a maximum and then gradually decreased in N application plots (N2-N6) during the growing season. Similar seasonal changes in SPAD values have been reported previously in rice (Jiang et al., 2012).

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Xiushui63: \( N_c = 5.31 W^{-0.5}, R^2 = 0.993 \) (2)
Hang43: \( N_c = 5.38 W^{-0.49}, R^2 = 0.997 \) (3)

The result showed that the \( N_c \) dilution curve \( (N_c = a_c W^{-b}) \) of Xiushui 63 was slightly different from that of Hang43. The parameter \( a_c \) is N\% when \( W = 1 \) t ha\(^{-1}\). The difference between the two varieties in critical N dilution curve slope \( (a_c) \) was larger than the parameter \( b \). This shows that when their dry matter was the same; \( N_c \) of Hang43 was larger than that of Xiushui63.

### 3. Correlation of NNI and N level

NNI correlated closely and positively with N level. The NNI at N4 level in 2011 and N5 level in 2012 was close to 1, so the N level was appropriate for both N treatments (Fig. 4). The NNI at N1 level gradually decreased from tillering stage to grain filling stage, but the trend was slowly changed with increasing N level. The NNI at N2 level slightly increased and was still less than 1 at booting stage. The NNI of N5 and N6 levels was close to or more than 1 all the time. The values of \( N_c \) and NNI adequately identified situations of deficient and non-deficient N nutrition in rice and can be used to quantify the level of rice N nutrition. The NNI is a reliable and precise indicator to characterize the N status of the rice crop throughout developmental stage. Moreover, it does not depend on cultivar, soil and weather conditions (Sheehy et al., 1998). However, NNI is rarely used in actual fertilizer management because it is time-consuming and expensive to determine the actual crop mass and N concentration. We suggest that the NNI could be used as a reference for
simpler procedures (e.g., chlorophyll measurements) to determine crop N status (Bélanger et al., 2003).

4. Correlation of SPAD and RSPAD values with NNI at tillering, booting and heading stage

NNI was significantly affected by N fertilization across leaf positions, varieties and developmental stages, which varied from 0.42 to 1.46 (Figs. 5 and 6). These values are within the range reported for rice (Siband et al., 2001). A value of NNI > 1 generally indicates excessive fertilization, while N deficiency increases with the decline of NNI from 1 to 0.2. A NNI value of 1 indicates optimal N status. NNI was positively related to SPAD values. The increase in NNI with increasing N fertilization has been previously reported in corn (Plénet and Cruz, 1997; Ziadi et al., 2008) and in wheat (Debaeke et al., 2006; Prost and Jeuffroy, 2007). This positive relationship varied across different developmental stages and leaf positions. The relationship between SPAD values of L1 or L2 and NNI was more stable across varieties and developmental stages ($0.56 < R^2 < 0.77$) than that of L3 or L4, and the relationship between SPAD values of L3 or L4 and NNI became closer at booting and heading stage ($0.75 < R^2 < 0.91$) than that of L1 or L2. Because 50% and 30% of total N was applied at the booting and heading stage, respectively, and the sensitivity of SPAD values of L3 and L4 to N was higher than L1 and L2 to N (Jiang et al., 2012; Li et al., 2007). These results not only proved that the lower leaf responded more to N supply than the upper leaf, but also that the L3 and L4 could be the ideal functional leaves for diagnosis of plant N nutrition in rice plants.

RSPAD values have been suggested to reduce the influence of the varieties, developmental stages and leaf positions on SPAD values (Wang et al., 2006; Lin et al., 2010; Jiang et al., 2012). In our study, RSPAD values were obtained by dividing the value in the test area by that in the N4 treatment (225 N kg ha$^{-1}$) in 2011 and in N5 treatment (300 N kg ha$^{-1}$) in 2012 that produced the largest shoot dry matter. Figs. 5 and 6 showed that RSPAD values were more closely related with NNI and the variability was reduced ($R^2$ ranging from 0.38 to 0.96) with increasing developmental stages and leaf positions in both years. However, the intercepts and slopes of the response lines of NNI as a function of RSPAD values for the different developmental stages and leaf positions were still different in 2011 and 2012. These results indicate that developmental stages and leaf positions are important factors influencing SPAD and RSPAD values.

Fig. 4. Dynamics of the nitrogen nutrient index (NNI) at different N application levels in rice in 2011 and 2012.
Since developmental stage has an important effect on SPAD values (Wang et al., 2006; Lin et al., 2010), we speculate that narrowing developmental stage would reduce the difference among varieties. Therefore, we limited our analysis to the relationship between NNI and SPAD values of L4 at booting stage. The NNI was positively related to SPAD values ($R^2_{L4} = 0.91^{**}$, in 2011, $R^2_{L4} = 0.89^{**}$, in 2012) and RSPAD values ($R^2_{L4} = 0.96^{**}$, in 2011, $R^2_{L4} = 0.91^{**}$, in 2012) of L4 at booting stage in both years (Figs. 5 and 6).

In Europe, NNI is recognized as a reference method for detecting N deficiency in wheat crop (Justes et al., 1997; Vouillot et al., 1998). Because of the practical issues of using NNI are for crop diagnosis in farmers’ fields, alternative methods are required to assess crop N status. Our results showed a significant relationship between NNI and SPAD values or RSPAD values ($p < 0.01$). When we limited our analysis to include just the RSPAD values and NNI at booting and heading stages, we found a more significant relation at all leaf positions and in all varieties (Figs. 5 and 6).

Fig. 5. NNI as a function of SPAD and RSPAD values of leaves at different positions (L1-L4) in Xiushui63 fertilized with various N rates at tillering, booting and heading stages in 2011. The L1-L4 separately represents the first, second, third and fourth fully expanded leaf. Solid lines represent linear regressions.
5. Correlation of SPAD values with grain yield, and that of RSPAD values and NNI with relative grain yield

The grain yield was positively related to SPAD values of L4 (Fig. 7). The relationship between grain yield and SPAD values of L4 at booting stage, expressed by a significant quadratic function, accounted for 82 and 72% of the variation in 2011 and 2012, respectively. However, the intercepts of the response curves of grain yield as a function of SPAD values in the two varieties were significantly different. Therefore, as we did for NNI, we used RSPAD values to study the relationship with relative grain yield. The relationship between relative grain yield and RSPAD values of L4 at booting stage, expressed by significant quadratic functions, accounted for 92 and 77% of the variation in 2011 and 2012, respectively. Similar results have been previously reported on corn and wheat (Waskom et al., 1996; Debaeke et al., 2006; Ziadi et al., 2008).

Experimental results indicated that RSPAD values could be useful to determine the yield response to additional N applications. However, as a diagnostic tool, RSPAD values also have limitations. The mathematical relationships
observed between relative grain yield and RSPAD values could: (i) vary across the developmental stages and leaf positions (Wang et al., 2006), which implies that the specific developmental stage and leaf position should be considered when RSPAD values were taken; (ii) reach a plateau at excessive N rates (Hussain et al., 2000; Li et al., 2011), which indicates the inability of SPAD meter to detect excess N treatment; and (iii) necessitate the establishment of an N-saturated treatment until the suitable developmental stage is reached for SPAD values (Wang et al., 2006).

For comparison, we also studied the relationship between relative grain yield and NNI, which was expressed by a quadratic function at booting stage. It accounted for 96 and 86% of the variation in 2011 and 2012, respectively. Based on the relationship, for a NNI > 0.96 in 2011 and NNI ≥ 1.03 in 2012, the relative grain yield was near 1.0 (Fig. 7). The relationship between relative grain yield and NNI was not affected by leaf positions. Furthermore, relative grain yield was more closely related to NNI than to RSPAD values. The relationship between relative grain yield and NNI also has other advantages over the relationship between relative grain yield and RSPAD values. It allows the detection of not only deficient N
Conclusions

We concluded that the critical N curve of Sheehy et al. (1998) can be applied to rice grown under the pedoclimatic conditions of Zhejiang, China. In addition, the NNI calculated from the critical N curve was a reliable indicator of the level of N status during the growing season. SPAD values and RSPAD values were significantly related to NNI, but the slope of the response lines varied with varieties, developmental stages, and leaf positions. Therefore, caution is needed in using these relationships to determine rice N status during the growing season. The SPAD and RSPAD values of L1 and L2 had a closer relationship with NNI than those of L3 and L4 at tillering stage, but it was reversed at booting and heading stages. The RSPAD values of L4 were more closely related to relative grain yield compared with the relationship between the SPAD values of L4 and grain yield at booting stage. The relationship between relative grain yield and NNI was stable across leaf positions and varieties and this relationship can be used to detect and quantify N deficiencies of rice plants at booting stage.

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*In Chinese.

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