Monitoring Leaf Nitrogen Status in Rice with Canopy Spectral Reflectance

Lihong Xue, Weixing Cao,* Weihong Luo, Tingbo Dai, and Yan Zhu

ABSTRACT

Nondestructive monitoring and diagnosis of plant N status is necessary for precision N management. The present study was conducted to determine if canopy reflectance could be used to evaluate leaf N status in rice (*Oryza sativa* L.). Ground-based canopy spectral reflectance and N concentration and accumulation in leaves were measured over the entire rice growing season under various treatments of N fertilization, irrigation, and plant population. Analyses were made on the relationships of seasonal canopy spectral reflectance, ratio indices, and normalized difference indices to leaf N concentration and N accumulation in rice under different N treatments. The results showed that at each sampling date, leaf N concentration was negatively related to the reflectance at the green band (560 nm) while positively related to ratio index, with the best correlation at jointing. However, the relationships between leaf N accumulation and reflectance at green band and ratio index were consistent across the whole growth period. The ratio of near infrared (NIR) to green (R_{810}/R_{560}) was especially linearly related to total leaf N accumulation, independent of N level and growth stage. Tests of the linear regression model with different field experiment data sets involving different plant densities, N fertilization, and irrigation treatments exhibited good agreement between the predicted and observed values, with an estimation accuracy of 96.69%, root mean square error of 0.7072, and relative error of −0.0052. These results indicate that the ratio index of NIR to green (R_{810}/R_{560}) should be useful for nondestructive monitoring of N status in rice plants.

Nitrogen fertilization is a major agronomic practice that affects the yield and quality of crop plants. The amount of N fertilizer needed often varies widely between fields and within fields while the amount of N is uniformly applied. For precision N application, nondestructive, instant, and reliable methods for assessing N status are necessary in field crops. However, rapid and accurate quantification of crop N status is often difficult and time-consuming. Current methods involve destructive sampling techniques and lengthy analysis with hazardous chemicals (Cataldo et al., 1975; Pettygrove et al., 1981). Varied N management practices result in differences in leaf area index (LAI), biomass, leaf chlorophyll, and tissue N concentrations that in turn contribute to the differences in canopy spectral reflectance (Hinzman et al., 1986). Therefore, it is theoretically promising to evaluate plant N status using canopy spectral properties. A relatively new procedure is to use a chlorophyll meter to assess the leaf N status from SPAD readings (Chubachi et al., 1986; Takebe et al., 1990; Wood et al., 1993). This new technology provides instantaneous results and has been demonstrated as an effective tool to schedule N fertilization for rice (*Oryza sativa* L.) on an *as-needed* basis (Turner and Jund, 1991; Peng et al., 1993). However, there are two factors that limit the use of SPAD meters for N fertilization. First, a within-field reference (usually an adequately fertilized area or strip within the field) is required to accurately quantify N deficiencies. Second, the SPAD meter collects point measurements from a single leaf on a single plant. Consequently, many leaves from a number of plants must be sampled to obtain a representative average value for a particular sampling date and to adequately assess the spatial variability. In contrast, remote sensing of canopy reflectance has the capability to sample a plant population or community rather than individual plants and to rapidly assess the spatial variability of a crop field.

The possibility of predicting crop N status using canopy reflectance spectra has been examined for major agronomic crops (Thomas and Oerther, 1972; Shibayama and Akiyama, 1986; Fernandez et al., 1994; Blackmer et al., 1996; Baret and Fourty, 1997; Wang et al., 1998; Shen et al., 2001). For a crop canopy, reflectance is low near the 480- and 680-nm region due to the strong absorption by chlorophyll and is high in the NIR region due to the microcellular structures in leaf material (Thomas and Oerther, 1972). Canopy spectral reflectance is determined not only by plant physiology and morphology, but also by soil characteristics, irradiation, observation angle, and atmospheric condition (Huete and Jackson, 1985). New leaves reflect more green light than older leaves because newer leaves are not uniformly green as they emerge from the whorl (Bausch and Duke, 1996). Chronological age did not influence NIR reflectance of leaves having the same nutrient treatment (Al Abbas et al., 1974). A decrease in LAI causes canopy reflectance in the NIR to decrease without any change in the reflectance properties of individual leaves (Colwell, 1974). Linear combination or some transformation of the spectral data from two or more wavebands called vegetation indices may yield a more accurate estimate of biophysical plant parameters such as leaf N concentration on dry matter basis (LNC) while reducing the impact of exogenous factors. Shibayama and Akiyama (1986) found that reflectance factors at 620 and 760 nm (regression with two variables) or 400, 620, and 880 nm (regression with three variables) gave the best correlations with rice LNC, and the relationship between the measured and predicted values was linear in spite of various types and cultivars of rice. Takebe et al. (1990) stated that green color intensity values and

Key Lab of Crop Growth Regulation, Ministry of Agric., Nanjing Agric. Univ., Nanjing, Jiangsu 210095, P.R. China. Support by National Natural Science Foundation of China (30030090) and State 863 Hi-tech Program (2002AA243011). Received 2 Feb. 2003. *Corresponding author (caow@njau.edu.cn).

Published in Agron. J. 96:135–142 (2004).

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Abbreviations: LAI, leaf area index; LNA, leaf nitrogen accumulation per unit ground area; LNC, leaf nitrogen concentration on dry matter basis; NIR, near infrared; RE, relative error; RMSE, root mean square error; RVI, ratio vegetation index (NIR/Red); R_x, reflectance at x nanometers.

total N content of the second leaf from the top were highly correlated. Inoue et al. (1998) showed a close relationship between the normalized difference of R_{1100} and R_{660} $[(R_{1100} - R_{660})/ R_{1100} + R_{660})]$ and leaf N accumulation per unit ground area (LNA) in rice when it was lower than 3 g m^{-2} and a combination of four spectral bands—R_{550}, R_{830}, R_{1650}, and R_{2200}—was useful for estimation of LNA. However, the regression equations established by these studies do not extrapolate to other sites and years as they depend on viewing and radiation geometries, canopy morphology, soil background, and the spectral characteristics of plant parts. So far, development of accurate and general models to monitor and predict LNC in rice from reflectance data is still a challenging task.

In this context, we initiated a series of field experiments under varied cultural conditions in the People's Republic of China involving N fertilization, water regime, transplanting density, and seedling age during the two rice growing seasons of 2001 and 2002. The objectives of the present study were to (i) characterize the ground-based canopy reflectance over the entire rice growing period, (ii) identify critical spectral reflectance indices for monitoring rice N status, and (iii) validate the critical reflectance index (linear regression model) for monitoring rice N status.

MATERIALS AND METHODS

Experiment Design

Experiment 1: Different Nitrogen Application Rates

The experiment was undertaken at the experiment station of Jiangsu Academy of Agricultural Science in 2001. The field soil was Gleyed paddy soil (Alfisols in U.S. taxonomy) with 16.7 g kg^{-1} organic matter (at 0–25 cm), 0.88 g kg^{-1} total N (at 0–25 cm), 43.85 mg kg^{-1} available phosphate (P_2O_5 at 0–25 cm), and 80.22 mg kg^{-1} available K (K_2O at 0–25 cm). A normal japonica rice cultivar, Wuxiangjing 9, was planted on 11 May and transplanted on 12 June with a density of 533.3×10^3 plants ha^{-1}. Nitrogen as urea was applied in four rates of 0 kg ha^{-1} N (N0), 135 (N1), 270 (N2), and 405 (N3), and the N application was distributed as 55% for preplanting basal, 20% for jointing dressing, and 25% for booting dressing, respectively. The topdressing of N was broadcasted on 1 August and 12 August, respectively. For all treatments, 135 kg ha^{-1} P_2O_5 {as monocalcium phosphate $[Ca(H_2PO_4)_2]$} and 210 kg ha^{-1} K_2O (as KCl) were incorporated into the soil before transplanting. The experiment was a randomized complete block design with three replications for each N treatment and 18-m^2 area for each plot.

Experiment 2: Different Nitrogen Rates and Water Regimes

The experiment was conducted at Jiangpu Experiment Station of Nanjing Agricultural University in 2002 on Gleyed paddy soil (Alfisols for U.S. taxonomy). The soil contained 20.9 g kg^{-1} organic matter (at 0–25 cm), 1.41 g kg^{-1} total N (at 0–25 cm), 12.03 mg kg^{-1} available phosphate (P_2O_5 at 0–25 cm), and 103.5 mg kg^{-1} available K (K_2O at 0–25cm). The japonica rice cultivar Wuxiangjing 9 was planted on 13 May and transplanted on 17 June with a density of 533.3×10^3 plants ha^{-1}. Five N rates (kg N ha^{-1}) as 0 (R1), 75 (R2), 150 (R3), 225 (R4), and 300 (R5) and two water regimes as intermittent irrigation system (W1) and shallow water system (W2) were applied, with N (as urea) distributed as 50% at preplanting, 10% at tillering, 20% at jointing, and 20% at booting. The experiment was a two-way factorial arrangement of treatments within the randomized complete block design, with three replications for each treatment and 30-m^2 area for each plot. The topdressing of N was made on 1 August and 12 August, respectively. For all treatments, 150 kg ha^{-1} P_2O_5 {as monocalcium phosphate $[Ca(H_2PO_4)_2]$} and 180 kg ha^{-1} K_2O (as KCl) were incorporated into the soil before transplanting.

Experiment 3: Different Varieties and Sowing Dates

The experiment was performed at Campus Experiment Station of Nanjing Agricultural University in 2002 on Gleyed paddy soil (Alfisols for U.S. taxonomy) with 14.2 g kg^{-1} organic matter (at 0–25 cm), 1.02 g kg^{-1} total N (at 0–25 cm), 81.36 mg kg^{-1} available phosphate (P_2O_5 at 0–25 cm), and 140.92 mg kg^{-1} available K (K_2O at 0–25cm). Two japonica rice cultivars, Koshihikari (early rice) and RR109 (late rice), and two sowing dates, 29 April (S1) and 6 June (S2), were applied. The seedlings of five-leaf age were transplanted respectively on 27 May and 27 June with a density of 655.9×10^3 plants ha^{-1}. The experiment design was the same as Exp. 2, with 10-m^2 area for each plot. For all treatments, 150 kg ha^{-1} N (as urea), 120 kg ha^{-1} P_2O_5 {as monocalcium phosphate $[Ca(H_2PO_4)_2]$}, and 120 kg ha^{-1} K_2O (as KCl) were applied and incorporated before transplanting. Nitrogen as urea was also applied at jointing (70 kg ha^{-1}) and at booting (50 kg ha^{-1}).

Experiment 4: Different Planting Densities and Seedling Ages

Two planting densities (plants ha^{-1}), 267×10^3 (D1) and 533×10^3 (D2), and two seedling ages, small seedling with 3.1 leaves at 3 wk old (A1) and medium seedling with 5.1 leaves at 5 wk old (A2), were applied, with the same experiment design as Exp. 2 and plot area of 15 m^2. The experiment site, year, soil property, cultivar, and transplanting date were detailed as Exp. 1. Before transplanting, 150 kg ha^{-1} N, 135 kg ha^{-1} P_2O_5 {as monocalcium phosphate $[Ca(H_2PO_4)_2]$}, and 210 kg ha^{-1} K_2O (as KCl) were incorporated into the soil for all treatments. Additional N applications were broadcasted on 1 August (50 kg ha^{-1}) and 12 August (70 kg ha^{-1}) for jointing dressing and booting dressing.

Measurements and Data Collection

Canopy Spectral Reflectance

The spectral reflectance of the plant canopy over the wavelength range of 447 to 1752 nm at 16 specific wavebands (approximate center wavelength = 460, 510, 560, 610, 660, 680, 710, 760, 810, 870, 950, 1100, 1220, 1480, 1500, and 1650 nm) was measured using a portable ground MSR16 radiometer (CROPSCAN, Rochester, MN). A data acquisition device (DLC Model 2000, CROPSCAN, Rochester, MN) equipped with sun angle cosine correction capacity was used to record reflectance data. Measurements were made at three sites over each plot, looking straight down from 2.0 m above the canopy. With a 31.1° field of view, the sensor viewed an area 1.0 m in diameter. Radiometer calibration was conducted daily with an opal glass diffuser using the two-point (2-Pt. Up/Dn) method (CROPSCAN, 2000). All spectral measurements were made on cloudless or near cloudless days at 1100 to 1400 h. Data were obtained on 10 different times or dates in Exp. 1, seven dates in Exp. 4, five dates in Exp. 2, and five dates in Exp. 3. These individual dates were selected to cover all major growth stages from active tillering to physiological maturity of rice.

Table 1. Training samples.

Experiment	Treatment†	Sampling date				
Exp. 1	N0, N3	4 July	16 July	31 July	22 Aug.	16 Sept.
	N1, N2	9 July	22 July	13 Aug.	30 Aug.	–
Exp. 2	W1R1, W1R3, W1R5	12 July	1 Aug.	2 Sept.	26 Sept.	
	W1R2, W1R4	24 July	20 Aug.	13 Sept.		
	W2R1, W2R3, W2R5	24 July	20 Aug.	13 Sept.		
	W2R2, W2R4	12 July	1 Aug.	2 Sept.	26 Sept.	

† N0 to N3 represent N rates 0, 135, 270, and 405 kg ha^{-1} N for Exp. 1 in 2001; R1 to R5 represent N rates 0, 75, 150, 225, 300 kg ha^{-1} N for Exp. 2 in 2002; W1, irrigation system; W2, shallow water system.

Plant Growth and Nitrogen Accumulation

Measurements of plant biomass components (leaf, stalk, and grain) and destructive LAI were obtained by randomly harvesting four to five hills from each plot. From each sample, a subsample of 10 to 20 tillers was randomly selected for measurement of green leaf blade area with the CI-203 (CID, Vancouver, WA) area meter. The components of the subsamples (leaf blades, stems including leaf sheaths, and heads) and the remaining large samples were put in separate bags, oven-dried at 70°C to constant weight, and then weighed. The LAI for each plot was calculated using specific leaf area (the ratio of green leaf area to dry weight). A random sample from all green leaves of each plot was taken for determination of total N concentration in tissues by the micro-Kjeldahl method. The LNA was calculated as the product of leaf N concentration per unit dry weight and leaf dry weight per unit ground area.

Data Analysis

Data collected from the experiments were mainly used for deriving the regression equations (training samples) and for validation of the derived equations (validation samples) under different cultural conditions. The training samples were data of different N levels, water regimes, and sample dates from Exp. 1 and Exp. 2 (Table 1) while all remaining data were used for validation.

The reflectance data were plotted with the wavebands and days after transplantation. Nitrogen treatment effects were analyzed quantitatively by comparing the means of agronomic parameters and reflectance spectra of each treatment through Duncan's multiple range test at a probability of 0.05. All possible ratio indices and normalized difference indices of NIR bands to visible bands were calculated from the raw reflectance data. Then regressions of reflectance and ratio indices and normalized difference indices to LNA were made using SAS software (SAS Inst., 1990). Linear or nonlinear models were fitted based on the plot patterns and best-fit R^2 values for the relationship. When no linearity exited, exponential, power, or quadratic models were attempted. An overwhelming proportion of the best spectra–biophysical relationships were either linear or power. On rare occasions, exponential or quadratic models provided only marginal increases in R^2 values, but these increases were generally insignificant. Hence, only linear or power relationships were reported. Analysis of covariance was used to test the significance of difference between different regression equations for each growth stage or N treatment. Then, leaf N status in the field-grown plants was predicted from the reflectance measurements based on the best regression model. The predicted and measured N status of the leaves was compared by univariate least-squares regression. The regressions were examined for precision (a correlation value close to 1.0 would indicate high precision) and accuracy (slope close to 1.0 when the intercept is 0 would indicate high accuracy) as described by Massart et al. (1988), and then the most reliable regression was identified as proposed model for prediction of leaf N status. For validation of the final model, root mean square error (RMSE) and relative error (RE) were calculated to test the goodness of fit between the predicted and observed values along with 1:1 plotting.

RESULTS AND DISCUSSION

Spectral Responses to Different Nitrogen Treatments

Rice growth was significantly affected by N application rates (Table 2), the plot with no N was characterized by lower LAI and biomass and a more erectophile appearance, and all of these changes were manifested in canopy reflectance. Reflectance spectra of the rice canopy under varying rates of N fertilization measured at

Table 2. Average leaf area index (LAI), leaf dry matter (LDM), leaf N concentration (LNC), and leaf N accumulation (LNA) for the different N treatments at different growth stages of rice in 2001 (Exp. 1).

Growth stage	N treatment	LAI	LDM	LNC	LNA
	kg ha^{-1} N	m^2 m^{-2}	g m^{-2}	g kg^{-1}	g m^{-2}
Tillering	0	0.81(0.05)†c‡	37.77(4.59)b	40.06(1.78)b	1.51(0.13)d
	135	1.04(0.09)b	46.71(5.08)a	41.94(1.09)b	1.95(0.22)c
	270	1.19(0.01)ab	51.77(7.67)a	44.73(1.07)a	2.31(0.18)b
	405	1.34(0.14)a	54.32(8.00)a	46.69(1.50)a	2.54(0.30)a
Jointing	0	3.25(0.33)d	159.8(3.3)d	30.32(0.94)c	4.85(0.17)d
	135	4.31(0.60)c	202.1(20.3)c	31.86(1.12)c	6.45(0.87)c
	270	5.05(0.47)b	237.1(17.1)b	34.40(1.21)b	8.14(0.75)b
	405	6.23(0.38)a	276.8(6.8)a	37.49(0.15)a	10.38(0.27)a
Heading	0	5.35(0.33)d	304.9(21.5)d	25.91(1.84)b	7.94(1.96)d
	135	7.79(0.30)c	419.1(36.7)c	27.56(0.32)b	11.55(1.24)c
	270	8.91(0.12)b	475.2(30.1)b	30.17(1.30)a	14.32(1.17)b
	405	9.63(0.07)a	541.8(26.1)a	32.26(0.59)a	17.47(0.77)a
Filling	0	3.69(0.51)d	289.4(31.5)c	23.70(2.28)b	6.89(1.46)d
	135	5.83(0.31)c	390.2(32.5)b	25.55(0.85)b	9.95(0.55)c
	270	6.42(0.25)b	426.7(8.6)b	28.17(0.69)a	12.02(0.35)b
	405	7.52(0.09)a	479.1(23.1)a	29.70(0.32)a	14.23(1.23)a

† Values in parentheses indicate the standard errors of means ($n = 3$).
‡ For a given development stage, values followed by different letters are significantly different at the $P < 0.05$ level (Duncan's Multiple Range Test).

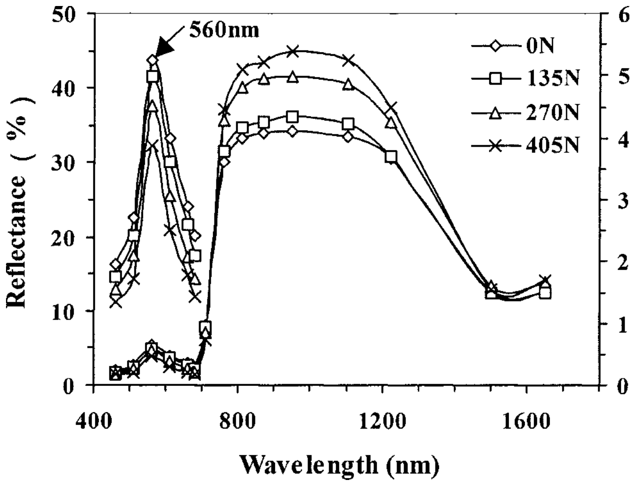

Fig. 1. Rice canopy reflectance with different N levels (0, 135, 270, and 405 kg ha^{-1} N) at tillering stage of Exp. 1.

Table 3. Average reflectance at 560, 810, 870, and 950 nm of rice with different N treatments at different growth stages in 2001 (Exp. 1).

Growth stage	N treatment	560 nm	810 nm	870 nm	950 nm
	kg ha^{-1} N	%			
Tillering	0	7.07a†	19.443c	19.913b	20.829b
	135	6.579a	21.512b	22.582b	22.617ab
	270	5.463b	23.235b	22.915ab	23.189ab
	405	5.016c	25.297a	25.669a	26.376a
Jointing	0	4.984a	31.093c	31.761b	32.196c
	135	4.432b	37.386b	38.894a	38.658b
	270	3.95c	39.14ab	39.482a	40.446ab
	405	3.465d	42.66a	43.759a	44.214a
Heading	0	3.478a	34.986b	37.226b	38.448b
	135	3.224a	39.869ab	42.374ab	43.843ab
	270	2.826b	43.555a	46.083a	47.522a
	405	2.416c	45.175a	47.797a	48.915a
Filling	0	3.639a	36.704b	37.838b	36.116b
	135	3.457a	37.568ab	39.369ab	37.667b
	270	3.156b	37.525ab	39.019ab	37.893ab
	405	3.084c	38.308a	40.405a	38.871a

† For a given development stage, values followed by different letters are significantly different at the $P < 0.05$ level (Duncan's Multiple Range Test).

the tillering stage (22 July 2001) are shown in Fig. 1. Reflectance decreased in the visible wavelength regions with increasing N supply and increased in the NIR wavelength regions. Similar spectral responses were observed on other dates of both 2001 and 2002. These spectral variations among N treatments resulted from changes in LNC, LAI, and biomass, all of which were altered by N treatments. The greatest differences at tillering are in the NIR region and between the N0 (0 kg ha^{-1} N) and N3 (405 kg ha^{-1} N) for 2001 and between the R0 (0 kg ha^{-1} N) and R4 (300 kg ha^{-1} N) for 2002. The differences between four N levels for 2001 in canopy reflectance at 610, 710, and 1100 nm were significant at 5% level while at 560, 810, 870, and 950 nm, the differences were significant at the 1% level (Table 3). These results are similar to those of Daughtry et al. (2000) and Wang et al. (1998). This indicates that varied N nutrition could be discriminated by specific spectral variables. The 560-nm region is strongly reflected by chlorophyll in green vegetation and is most sensitive to chlorophyll concentration (Thomas and Gausman, 1977; Gitelson and Merzlyak, 1994a, 1994b); thus, it is closely associated with N concentration in leaf tissues due to the close link between N concentration and chlorophyll concentration (Yoder and Pettigrew-Crosby, 1995). In addition, the data indicated that the differentiating ability for N treatments at 560 nm was stronger than the NIR bands throughout growth season (Table 3). Therefore, 560 nm was selected as the sensitive band to assess N status in rice plant.

Relationships of Canopy Reflectance to Leaf Nitrogen Concentration on Dry Matter Basis and Leaf Nitrogen Accumulation per Unit Ground Area

Higher N rate produced rice plants with higher leaf N concentration (Table 2). Thus, reflectance in a N-sensitive band (560 nm) should be a good indicator of N status of the rice plants. The canopy reflectance at 560 nm was negatively related to LNC at each sampling date (Fig. 2A), in agreement with previous research (Thomas and Oerther, 1972; Blackmer et al., 1994; Gitelson et al., 1996). When considering all of the data in a whole, it seems that a positive relationship existed before heading (days after transplanting = 79) and a negative relationship existed after heading (Fig. 2A). The LNC decreased gradually with plant growth, from the average value of 43.36 g kg^{-1} at tillering to 26.78 g kg^{-1} at filling for all treatments. The largest LNC difference between the zero-N treatment and high-N treatment (405 kg ha^{-1} N) occurred at jointing, with a value of 7.17 (Table 2), 40% of the difference of zero-N treatment of the whole growth cycle. It is clear that the LNC differences between N treatments were overshadowed by the differences between growth stages and the positive relationship with growth stage in Fig. 2A was produced by the combination of all preheading data points. Analysis of covariance showed that there were significant differences among the regression slopes of LNC vs. reflectance at 560 nm for different growth stages ($P < 0.01$, analysis of covariance). This indicates that growth stage is very important when trying to determine N status in rice or other small-grain crops. Consequently, separate regression equations were established for each growth stage, listed in Table 4. With the progress of rice growth, the slope of reflectance at 560 nm vs. LNC regression increased, with the best R^2 (0.82) at jointing.

However, when the reflectance at 560 nm was related to LNA, a consistent relationship was obtained throughout the whole growth season, independent of N treatment (Fig. 2B). Reflectance at 560 nm nonlinearly decreased with increasing LNA. Compared with LNC, LNA relatively enlarged the differences between the treatments so that it became more sensitive to N fertilization rates and exhibited differences corresponding to N rate on each sampling date (Table 2). Moreover, LNA is the product of LNC and leaf dry matter per unit ground area (LDM), which is the product of LAI and specific leaf weight (SLW), as expressed in the following equation:

$$LNA = LNC \times LDM = LNC \times SLW \times LAI$$

Thus, LNA includes not only the impact of leaf area,

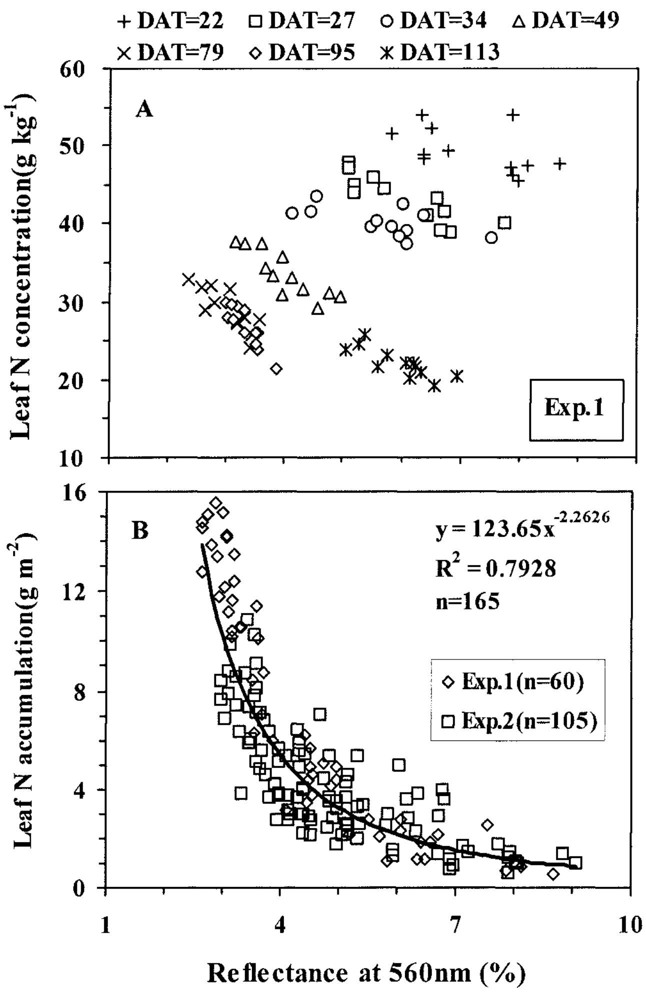

Fig. 2. Relationship of canopy reflectance at 560 nm to (A) leaf N concentration and (B) leaf N accumulation in rice with different N treatments over the growth period. DAT, days after transplanting.

but also the impact of SLW that is associated with the leaf structure and components. The spectra obtained by remote sensing reflect the complex information of whole vegetation, including the leaves, stems, spikes, soil, and other backgrounds. Thus, estimation of canopy-based variables such as LNA may be more proper than leaf-based variables such as LNC using canopy spectral data. This supports the idea that LNA is a good indicator of N status of the plant canopy in the remote sensing system, in which LNA could be estimated for N diagnosis and growth prediction. Yet the reflectance at 560 nm approached the asymptote when LNA was greater than 9 g m^{-2}. This indicates that canopy reflectance measurement at a single band has limited usefulness for estimation when LNA is high. Daughtry et al. (2000) also found that attempts to assess plant N status based on canopy reflectance in a single band often will be confounded by the variability in background reflectance and/or LAI. To assess leaf N status from remotely sensed observations, spectral indices are needed that are sensitive to leaf chlorophyll concentration and that minimize variations in canopy reflectance associated with background reflectance and LAI.

Table 4. Regression parameters for the linear relationships between leaf N concentration (LNC) and reflectance at 560 nm (R_{560}) and ratio index R_{810}/R_{560} at different growth stages of rice.

Variable	Growth stage	Intercept	Slope	R^2	*n*	SEE†
R_{560}	tillering	61.273	−2.986	0.74	12	1.622
	jointing	52.919	−4.859	0.82	12	1.219
	heading	45.969	−5.629	0.67	12	1.656
	filling	56.556	−8.931	0.78	12	1.488
R_{810}/R_{560}	tillering	31.426	3.088	0.86	12	1.177
	jointing	21.052	1.247	0.87	12	1.152
	heading	19.190	0.703	0.69	12	1.596
	filling	2.650	2.129	0.79	12	1.271

† SEE, standard error of the estimates.

Relationships of Ratio Indices and Normalized Difference Indices to Leaf Nitrogen Concentration on Dry Matter Basis and Leaf Nitrogen Accumulation per Unit Ground Area

To identify N prediction models applicable to the whole growth period, all regressions between ratio indices and normalized difference indices and leaf N status were examined for precision and accuracy. The results showed that all the ratio indices were better related to LNA than normalized difference indices, and average R^2 increased from 0.75 to 0.87 (Table 5). Only those ratio indices obtained from NIR and 460, 510, 560 nm had high R^2 (>0.90). So, normalized difference indices were discarded, and only ratio indices were used for further analysis. Precision and accuracy analysis showed that only a few indices such as R_{810}/R_{560}, R_{870}/R_{460}, R_{760}/R_{560}, R_{810}/R_{460}, and R_{760}/R_{560} provided good precision and accuracy, with R_{810}/R_{560} as the best index (Table 6). Although the coefficients of determination (R^2) of R_{1100}/R_{460}, R_{870}/R_{460}, and R_{950}/R_{460} to LNA were equal to R_{810}/R_{560} (0.91), their accuracy and precision were less than R_{810}/R_{560} and so not applicable for actual prediction (Table 5 and 6). The best indices are not necessarily those with the highest R^2 but should be those with both high R^2 and high precision and accuracy. Thus, the ratio index of R_{810}/R_{560} was the best index to estimate leaf N status under varied N rates, agreeing with Inada's results on leaf chlorophyll estimation (Inada, 1985).

Figure 3A shows the relationship between the ratio index R_{810}/R_{560} and LNC in our experiment. It appeared that the relationship was negative when considering all of the data before heading and positive after heading (days after transplanting = 79). A similar result was also found by Inoue et al. (1998). The relationship between the ratio index R_{810}/R_{560} and LNC for the vegetative period may be due to the fact that the differences between growth stages overshadowed the differences between N

Table 5. The correlation coefficients of ratio indices (ratio) and normalized difference indices (ND) to leaf N accumulation (LNA) in rice.

Ratio	760	810	870	950	1100	1220	ND	760	810	870	950	1100	1220
460	0.89	0.90	0.91	0.91	0.91	0.83	460	0.75	0.75	0.74	0.72	0.72	0.65
510	0.89	0.90	0.89	0.89	0.90	0.85	510	0.78	0.77	0.77	0.74	0.74	0.68
560	0.90	0.91	0.90	0.89	0.83	0.85	560	0.82	0.82	0.81	0.79	0.78	0.71
610	0.88	0.88	0.89	0.89	0.90	0.83	610	0.78	0.78	0.78	0.76	0.76	0.70
660	0.86	0.86	0.88	0.88	0.88	0.82	660	0.75	0.76	0.75	0.73	0.73	0.68
680	0.84	0.86	0.86	0.86	0.86	0.80	680	0.74	0.74	0.73	0.72	0.72	0.66
710	0.88	0.89	0.86	0.89	0.90	0.83	710	0.82	0.82	0.82	0.80	0.80	0.72

Table 6. The precision and accuracy of ratio indices for estimating leaf N accumulation (LNA) in rice.

Statistics	Ratio	460 nm	510 nm	560 nm	610 nm	660 nm	680 nm	710 nm
Precision	760 nm	0.890	0.888	0.901	0.872	0.858	0.836	0.874
	810 nm	0.903	0.898	0.916	0.882	0.864	0.857	0.882
	870 nm	0.913	0.889	0.894	0.891	0.880	0.863	0.891
	950 nm	0.910	0.884	0.895	0.888	0.878	0.860	0.889
	1100 nm	0.914	0.894	0.809	0.898	0.883	0.865	0.900
	1220 nm	0.832	0.851	0.852	0.834	0.822	0.799	0.827
Accuracy	760 nm	0.967	0.985	0.974	0.967	0.978	0.985	0.952
	810 nm	0.975	0.980	0.988	0.962	0.965	0.967	0.944
	870 nm	0.963	0.981	0.948	0.952	0.965	0.949	0.949
	950 nm	0.954	0.987	0.963	0.965	0.977	0.958	0.967
	1100 nm	0.844	0.889	0.974	0.973	0.991	0.997	0.976
	1220 nm	0.895	0.926	0.918	0.947	0.948	0.947	0.928

treatments while the relationship for the ripening period may be due to the fact that rice canopies lose greenness while maintaining a consistent amount of biomass during the senescence stage. A significantly positive relationship existed when considering each sampling date, and the relationship varied with the progress of growth stage and so cannot be incorporated into an individual equation (Fig. 3A). Consequently, to derive LNC from ratio index measurements, a separate linear equation for each growth stage had to be considered (Table 4). The relationships between LNC and the ratio index were better than those between LNC and reflectance at 560 nm. Jointing stage was the best time for LNC estimation, with the highest R^2 of 0.87 and the lowest standard estimated error of 1.152.

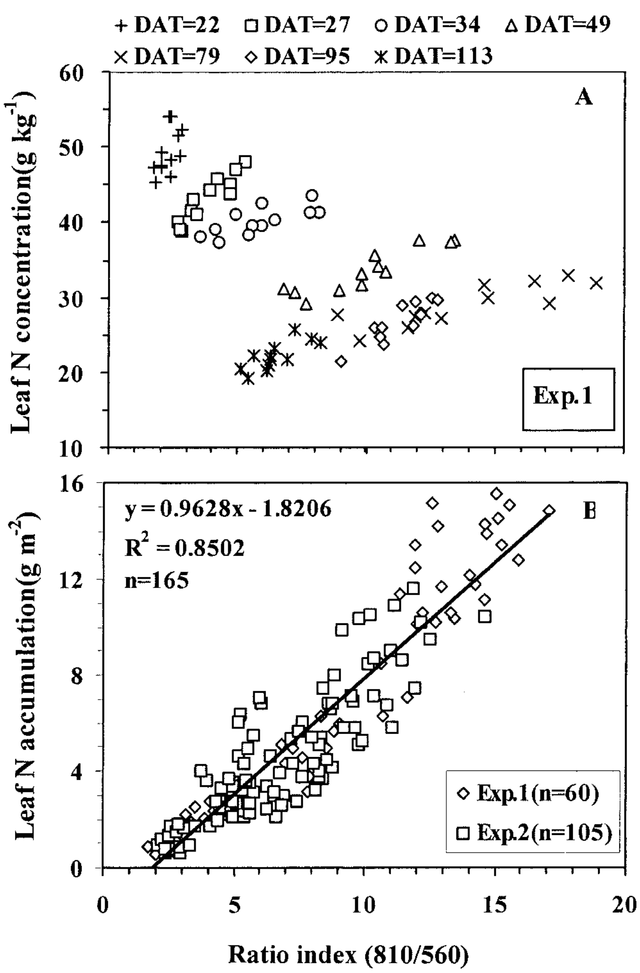

Fig. 3. Relationship of ratio index of near infrared (NIR, 810 nm) to green band (560 nm) to N (A) concentration and (B) accumulation in leaves of rice with different N treatments over the growth period. DAT, days after transplanting.

However, when considering the relationships between ratio index R_{810}/R_{560} and LNA, differences among growth stages in the slope of the relationship were not significant. Consequently, a single linear equation was appropriate to derive LNA values from ratio index R_{810}/R_{560} for the entire growth season (Fig. 3B). The relationship between R_{810}/R_{560} and LNA was linear and highly significant ($R^2 = 0.8502$) as follows:

$$\text{LNA} = 0.9628 \times R_{810}/R_{560} - 1.8206 \ (n = 165)$$

This result supports the previous observation that ratio of NIR to green had a good relationship with chlorophyll per unit land area and N accumulation (Shibayama and Akiyama, 1986; Takihashi et al., 2000). Similarly in wheat, LNA was found to be linearly related to ratio vegetation index (RVI, NIR/red) (Hinzman et al., 1986). However, the present data show that both the accuracy and precision for prediction of LNA using RVI were lower than that of NIR/green (R_{810}/R_{560}) (Table 6). Aoki and Totsuka (1985) also reported that the ratio R_{880}/R_{550} may be more useful than the ratio R_{800}/R_{680} for estimating the amount of chlorophyll per unit land area when the plant canopy consists of a single species as in agricultural fields. It seems that the present finding can be explained from underling physiological basis. Canopy reflectance is mainly influenced by LAI, background reflectance, and leaf chlorophyll concentration. To assess crop N status from remotely sensed observations, spectral indices should be sensitive to leaf chlorophyll with background reflectance and LAI. Ratio vegetation index minimizes contributions of background reflectance but is relatively insensitive to chlorophyll concentration whereas NIR/green is responsive to both leaf chlorophyll concentration and background reflectance (Daughtry et al., 2000; Gitelson and Merzlyak, 1994a; Gitelson et al., 1996). The green band (560 nm) is very sensitive to N rate (Thomas and Oerther, 1972; Al Abbas et al., 1974; Blackmer et al., 1994; Wang et al., 1998) while 810 nm, as a NIR band related to leaf structure, is possibly acting to normalize the index with respect to leaf properties and provide a baseline for the N-sensitive band. This would increase the precision and accuracy of NIR/green compared with RVI. Also, the ratio between reflectance of a stress-sensitive band and a stress-insen-

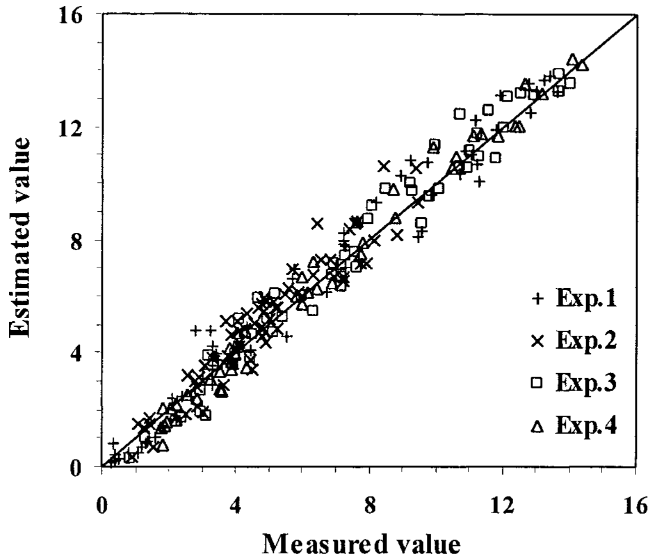

Fig. 4. Comparison of estimated with measured leaf N accumulation in rice from different experiments. Exp. 1, different N application rates in 2001; Exp. 2, different N rates and water regimes; Exp. 3, different varieties and sowing dates; Exp. 4, different planting densities and seedling ages.

sitive band could correct the variation of canopy reflectance resulting from the variation in irradiance, leaf orientation, irradiance angles, and shading (Tarpley et al., 2000).

However, some scatter exists in Fig. 3B. Some of this scatter may be attributed to: (i) the amount of soil seen by the radiometer from a nadir view angle varied among the N treatments and development stages due to LAI differences, (ii) changes of soil background created changes in canopy reflectance that were not crop related, (iii) filter bandwidth may be wider than desired, and (iv) changes in solar azimuth and zenith angles occurred during the 1 to 2 h required to measure reflectance of all plots on each date (Hinzman et al., 1986).

Test of the Linear Regression between Leaf Nitrogen Accumulation per Unit Ground Area and R_{810}/R_{560}

To be useful for remote sensing, an algorithm for predicting N status should be applicable over a wide range of vegetation types. To test the above linear model, four data sets from the different experiments were used to predict LNA. As expected, the validation results indicated a good agreement between the predicted and observed values (Fig. 4) although the data sets involved a number of cultural conditions. The estimation precision and accuracy for individual data sets were all above 0.90, RMSE all below 0.8, and RE below 5% (Table 7). For all the combined data sets, the model gave estimation precision of 0.9669, accuracy of 0.9798, RMSE of 0.7072, and RE of −0.0052. Thus, the ratio of NIR to green band (R_{810}/R_{560}) can be considered a reliable index for estimating LNA in rice plant.

The actual robustness of this methodology and its use needs to be verified in other sites although its performance was good in this study. The method should be

Table 7. Test results for different validation data sets.

Validation data set	Precision	Accuracy	RMSE†	Relative error
Exp. 1	0.9724	0.9761	0.5499	0.0350
Exp. 2	0.9090	0.9473	0.7721	−0.0398
Exp. 3	0.9696	0.9865	0.6768	−0.0222
Exp. 4	0.9855	0.9839	0.5399	−0.0335
All data sets	0.9669	0.9768	0.7072	−0.0052

† RMSE, root mean square error.

valuable for other crops as well, and further work should focus on developing critical N levels for rice needs and establishing a system for predicting optimum rates of N fertilization for rice during the whole growing season. Research on wheat has shown that critical LNA values at Growth Stage 30 vary greatly between environments (48 kg ha^{-1} reported by Roth et al., 1989; and 95 kg ha^{-1} reported by Baethgen and Alley, 1989) because the same LNA values can be determined by having a low LNC but a high biomass or a high LNC and low biomass while LNC values are consistent across environments (35.0 g kg^{-1} reported by Roth et al., 1989; 36.0 g kg^{-1} reported by Fox et al., 1994; and 39.5 g kg^{-1} reported by Baethgen and Alley, 1989). Thus, practical use of LNA for monitoring N concentration in crop plants needs a simultaneous estimation of growth status such as leaf dry weight and leaf area. Nonetheless, direct LNC estimation with canopy reflectance spectra should be strengthened, and the right set of spectral bands, the combination of which will enhance sensitivity to N status and reduce responsibility to background and canopy structure effects, should be analyzed. Moreover, a careful analysis should be performed to investigate the effects of band center location and bandwidth.

CONCLUSION

The strong correlations between canopy reflectance spectra and LNC and LNA indicate that the reflectance procedure has promise for assessing N status in rice. Based on reflectance, 560 nm was proven to be the best wavelength to separate N treatment differences. Reflectance at 560 nm was negatively correlated with LNC for each sampling date, and the relationship varied with growth stage. While the best-fit relationship to LNA was a power function for the whole growing season, sensitivity was lost when LNA was greater than 9 g m^{-2}. The results suggested that the predictive value of a single band is limited when rice N status is high. Thus, ratio index and normalized difference index were introduced. Results of correlation analysis showed that ratio indices were better related to LNC and LNA than normalized difference indices. The ratio index of NIR/green (R_{810}/R_{560}) was the best index as determined by precision and accuracy analysis and was positively related to LNC for each single growth stage, with the best prediction at jointing. However, the NIR/green ratio was linearly related to LNA, independent of growth stage and N treatment. Testing with different dependent data sets showed a good relationship between estimated and actual values. Thus, this relationship seems to be promising as a practical and usable technique for tissue monitoring and precision management of N nutrition in a rice crop. Nevertheless, still more accurate estimation of N status may be needed for determination of crop N fertilizer need.

REFERENCES

Al Abbas, A.H., R. Barr, J.D. Hall, F.L. Crane, and M.F. Baumgardner. 1974. Spectra of normal and nutrient deficient maize leaves. Agron. J. 66:16–20.

Aoki, M., and T. Totsuka. 1985. An evaluation of chlorophyll content and leaf area index of canopies based on spectral reflection characteristics. (In Japanese, with English summary.) Res. Rep. Natl. Inst. Environ. Stud., Jpn. 82:137–147.

Baethgen, W.E., and M.M. Alley. 1989. Optimizing soil and fertilizer nitrogen use by intensively managed winter wheat: II. Critical levels and optimum rates of nitrogen fertilizer. Agron. J. 81:120–125.

Baret, F., and T.H. Fourty. 1997. Radiometric estimates of N status of leaves and canopies. p. 201–228. *In* G. Lemaire (ed.) Diagnosis of the N status in crops. Springer-Verlag, Berlin.

Bausch, W.C., and H.R. Duke. 1996. Remote sensing of plant nitrogen status in corn. Trans. ASAE 39(5):1869–1875.

Blackmer, T.M., J.S. Schepers, and G.E. Varvel. 1994. Light reflectance compared with other N stress measurements in corn leaves. Agron. J. 86:934–938.

Blackmer, T.M., J.S. Schepers, G.E. Varvel, and E.A. Walter-Shea. 1996. Nitrogen deficiency detection using shortwave radiation from irrigated corn canopies. Agron. J. 88:1–5.

Cataldo, D.A., M. Haroon, L.E. Schrader, and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6(1):71–80.

Chubachi, T., I. Asano, and T. Oikawa. 1986. The diagnosis of N nutrition of rice plants using chlorophyll meter. Jpn. J. Soil Sci. Plant Nutr. 57:190–193.

Colwell, J.E. 1974. Vegetation canopy reflectance. Remote Sens. Environ. 3(3):175–183.

CROPSCAN. 2000. Data logger controller, user's guide and technical reference. CROPSCAN, Rochester, MN.

Daughtry, C.S.T., C.L. Walthall, M.S. Kim, E.B. de Colstoun, and J.E. McMurtrey III. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sens. Environ. 74:229–239.

Fernandez, S., D. Vidal, E. Simon, and L.S. Sugranes. 1994. Radiometric characteristics of *Triticum aestivum* cv. Astral under water and N stress. Int. J. Remote Sens. 15:1867–1884.

Fox, R.H., W.P. Piekielek, and K.M. Macneal. 1994. Using a chlorophyll meter to predict nitrogen fertilizer needs of winter wheat. Commun. Soil Sci. Plant Anal. 25 (3–4):171–181.

Gitelson, A.A., Y.J. Kaufman, and M.N. Merzlyzak. 1996. Use of a green channel in the remote sensing of global vegetation from EOS-MODIS. Remote Sens. Environ. 58:289–298.

Gitelson, A.A., and M.N. Merzlyak. 1994a. Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. J. Plant Physiol. 143:286–292.

Gitelson, A.A., and M.N. Merzlyak. 1994b. Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and male leaves. J. Photochem. Photobiol., B 22:247–252.

Hinzman, L.D., M.E. Bauer, and C.S.T. Daughtry. 1986. Effects of N fertilization on growth and reflectance characteristics of winter wheat. Remote Sens. Environ. 19:47–61.

Huete, A.R., and R.D. Jackson. 1985. Spectral response of a plant canopy with different soil backgrounds. Remote Sens. Environ. 17:37–53.

Inada, K. 1985. Spectral ratio of reflectance for estimating chlorophyll content of leaf. Jpn. J. Crop Sci. 54:261–265.

Inoue, Y., M.S. Moran, and T. Horie. 1998. Analysis of spectral measurements in paddy field for predicting rice growth and yield based on a simple crop simulation model. Plant Prod. Sci. 1:269–279.

Massart, D.L., B.G.M. Vandeginste, S.M. Deming, Y. Michotte, and L. Kaufman. 1988. Chemometrics: A textbook. Elsevier, Amsterdam.

Peng, S.B., F.V. Garcia, R.C. Laza, and K.G. Cassman. 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agron. J. 85:987–990.

Pettygrove, G.S., T.E. Kearney, S.W. Kite, R.L. Salisbery, S.C. Scardaci, and J.F. Williams. 1981. Tissue analysis to determine nitrogen status of wheat in California. p. 187. *In* 1981 agronomy abstracts. ASA, Madison, WI.

Roth, G.W., R.H. Fox, and H.G. Marshall. 1989. Plant tissue tests for predicting nitrogen fertilizer requirements of winter wheat. Agron. J. 81:502–507.

SAS Institute. 1990. SAS user's guide: Statistics. Version 6.12. SAS Inst., Cary, NC.

Shen, Y.A., J.C. Lo, and S.P. Cheng. 2001. Development of remote sensing techniques to identify N status of paddy rice. (In Chinese, with English abstract.) Chin. J. Agrometeorol. 7:23–31.

Shibayama, M., and T.A. Akiyama. 1986. A spectroradiometer for field use: VI. Radiometric estimation for chlorophyll index of rice canopy. Jpn. J. Crop Sci. 55:433–438.

Takebe, M., T. Yoneyama, K. Inada, and T. Murakami. 1990. Spectral reflectance ratio of rice canopy for estimating crop N status. Plant Soil 122:295–297.

Takihashi, W., V. Nguyen-Cong, S. Kawaguchi, M. Minamiyama, and S. Ninomiya. 2000. Statistical models for prediction of dry weight and N accumulation based on visible and near-infrared hyperspectral reflectance. Plant Prod. Sci. 3:377–386.

Tarpley, L., K.R. Reddy, and G.F. Sassenrath-Cole. 2000. Reflectance indices with precision and accuracy in predicting cotton leaf nitrogen concentration. Crop Sci. 40:1814–1819.

Thomas, J.R., and H.W. Gausman. 1977. Leaf reflectance vs. leaf chlorophyll and carotenoid concentration for eight crops. Agron. J. 69:799–802.

Thomas, J.R., and G.F. Oerther. 1972. Estimating nitrogen content of sweet pepper leaves by reflectance measurements. Agron. J. 64:11–13.

Turner, F.T., and M.F. Jund. 1991. Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. Agron. J. 83:926–928.

Wang, K., Z.Q. Shen, and R.C. Wang. 1998. Effects of N nutrition on the spectral reflectance characteristics of rice leaf and canopy. J. Zhejiang Agric. Univ. 24:93–97.

Wood, C.W., D.W. Reeves, and D.G. Himelrick. 1993. Relationships between chlorophyll meter reading and leaf chlorophyll concentration, N status, and crop yield: A review. Proc. Agron. Soc. N.Z. 23:1–9.

Yoder, B.J., and R.E. Pettigrew-Crosby. 1995. Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400–2500nm) at leaf and canopy scales. Remote Sens. Environ. 53:199–211.