Analysis of Common Canopy Reflectance Spectra for Indicating Leaf Nitrogen Concentrations in Wheat and Rice

Yan Zhu, Yongchao Tian, Xia Yao, Xiaojun Liu and Weixing Cao

(Hi-Tech Key Lab of Information Agriculture of Jiangsu Province, Nanjing Agricultural University, 1 Weigang Road, Nanjing, Jiangsu 210095, P. R. China)

Abstract: Non-destructive monitoring and diagnosis of plant nitrogen (N) concentration are of significant importance for precise N management and productivity forecasting in field crops. The present study was conducted to identify the common spectra wavebands and canopy reflectance spectral parameters for indicating leaf nitrogen concentration (LNC, mg N g\textsuperscript{-1} DW) and to determine quantitative relationships of LNC to canopy reflectance spectra in both rice (\textit{Oryza sativa} L.) and wheat (\textit{Triticum aestivum} L.). Ground-based canopy spectral reflectance and LNC were measured with seven field experiments consisting of seven different wheat cultivars and five different rice cultivars and varied N fertilization levels across three growing seasons for wheat and four growing seasons for rice. All possible ratio vegetation indices (RVI), difference vegetation indices (DVI), and normalized difference vegetation indices (NDVI) of key wavebands from the MSR16 radiometer were calculated. The results showed that LNC of wheat and rice increased with increasing N fertilization rates. Canopy reflectance, however, was a more complicated relationship under different N application rates. In the near infrared portion of the spectrum (760−1220 nm), canopy spectral reflectance increased with increasing N supply, whereas in the visible region (460−710 nm), canopy reflectance decreased with increasing N supply. For both rice and wheat, LNC was best estimated at 610, 660 and 680 nm. Among all possible RVI, DVI and NDVI of key bands from the MSR16 radiometer, NDVI(1220, 610) and RVI(1220, 610) were most highly correlated to LNC in both wheat and rice. In addition, the correlations of NDVI(1220, 610) and RVI(1220, 610) to LNC were found to be higher than those of individual wavebands at 610, 660 and 680 nm in both wheat and rice. Thus LNC in both wheat and rice could be indicated with common wavebands and vegetation indices, but separate regression equations are necessary for precisely describing the dynamic change patterns of LNC in wheat and rice. When independent data were fit to the derived equations, the root mean square error (RMSE) values for the predicted LNC with NDVI(1220, 610) and RVI(1220, 610) relative to the observed values were 10.50% and 10.52% in wheat, and 13.04% and 12.61% in rice, respectively, indicating a good fit. These results should improve the knowledge on non-destructive monitoring of leaf N status in cereal crops.

Key words: Canopy reflectance spectra, Leaf nitrogen concentration, NDVI, Rice, RVI, Wheat.

Nitrogen (N) is a major essential element for plant growth and yield formation in agronomic crops. Rice (\textit{Oryza sativa} L.) and wheat (\textit{Triticum aestivum} L.) are among the largest consumers of N fertilizers (Janssen, 1998). Thus, real-time monitoring and assessing of tissue N status in rice and wheat are of significant importance for guiding precision diagnosis and efficient management of plant N nutrition as well as realizing accurate prediction of yield formation and N flow in agricultural production systems (Raun and Johnson, 1999; Zhang and Ma, 2000; Li et al., 2003; Liu et al., 2003).

Traditional measurement methods of crop N status normally depended on plant sampling from the field and analytic assay in the lab (Roth and Fox, 1989; Li et al., 2003). The results from this protocol are relatively reliable, but are weak in temporal and spatial scale to meet the needs of real-time, fast and non-destructive monitoring and diagnosis of plant N status.

Several new methods have been proposed for non-destructive estimation of plant N nutrition, assuming that plant N status can be determined by leaf color charts, chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc.), reflectance spectra, and chlorophyll fluorescence (Filella et al., 1995; Ntamatungiro et al., 1999; Johnkutty et al., 2000). Many studies have indicated that these procedures are potentially useful for diagnosis of plant N nutrition and N fertilizer recommendation for crop (Li et al., 2003). Remote sensing technique has provided a new means for non-destructive and real-time monitoring of crop N status, and is exhibiting a promising prospect in growth monitoring, nutrition diagnosis, and yield estimating in field crops (Everitt et al., 1987; Jensen and Lorenzen, 1990; Filella et al., 1995; Thenkabail et al., 2000; Jongsgaap and Booij, 2004).

As early as 1972, Thomas and Oerther (1972) found that the leaf nitrogen concentration (LNC, mg N
Monitoring Leaf Nitrogen in Wheat and Rice

Zhu et al. —— Monitoring Leaf Nitrogen in Wheat and Rice

401

g DW of green pepper was closely associated with the reflectance characters within the wavebands of 550–675 nm in leaves, with the errors less than 7% between observed and predicted LNC. This implied that plant spectral analysis could possibly be used for fast, simple, accurate, and non-destructive monitoring of plant N nutrition. Since then, many studies have been focused on the sensitive spectral bands and monitoring methods for tissue N concentrations in crop plants (Everitt et al., 1987; Jensen and Lorenzen, 1990; Filella et al., 1995; Yoder and Pettigrew-Crosby, 1995; Blackmer et al., 1996; Stone et al., 1996; Lee et al., 2000; Curran et al., 2001; Xue et al., 2004). Everitt et al. (1987) indicated that there was high correlation between the reflectance at 500–750 nm and LNC in weeds and ornamentals, and suggested that reflectance ratio of 550–600 nm to 800–900 nm could be used for monitoring plant N status. Blackmer et al. (1996) reported that the reflectance near 550 nm could determine the differences among N treatments in maize and proposed 550–710 nm as N sensitive band. Wang et al. (1993) proposed 760–900 nm, 630–660 nm and 530–560 nm as sensitive spectral bands for N nutrition diagnosis in rice. Stone et al. (1996) suggested that total N concentration in wheat plant could be estimated with the spectral index based on the combination of two spectral bands at 671 and 780 nm. LNC of wheat could be derived by the canopy spectral indices based on the reflectance of 660 nm and 460 nm (Xue et al., 2004). These studies indicate that the proper spectral bands for LNC may change with crop species and growth conditions, but the spectral reflectance is useful for indicating N concentration in rice or wheat plant. However, information about using common sensitive wavebands and spectral indices for efficient monitoring of N concentration in both rice and wheat crops is lacking.

The objectives of the present study were: (1) to conduct a comprehensive analysis on the relationships between canopy reflectance spectra and LNC in both rice and wheat, (2) to identify the common spectra bands and spectral parameters indicating LNC in both rice and wheat crops, and (3) to determine quantitative equations applicable for LNC monitoring in rice and wheat crops. The expected results will improve the knowledge on non-destructive monitoring of leaf N status in cereal crops.

Materials and Methods

1. Experiment design

The data used in the present study were obtained from three experiments for wheat (Triticum aestivum L.) and four experiments for rice (Oryza sativa L.), respectively, each crop involving different years, cultivars and N fertilization rates.

(1) The experiments with wheat

Exp. 1: different N rates and varieties. The experiment was carried out at the campus experiment station of Nanjing Agricultural University, China (118°50' E, 32°02' N) for a single season from 2001 to 2002. The soil of the field was classified as Gleyed paddy soil (Alfisols in U.S. taxonomy) with 12.1 g kg−1 organic matter, 1.3 g kg−1 total N, 23.3 mg kg−1 available phosphate (extracted with 0.5 M NaHCO3), 97.2 mg kg−1 available potassium (0–25 cm soil depth, extracted with 1 M NH4OAc). Two winter wheat cultivars, Huaimai 18 and Xumai 26, were sown on 8 Nov. 2001 with a density of 150 plants m−2. Urea N fertilizer was applied at 0, 120, 210 and 300 kg N ha−1. Half N was applied as pre-sowing basal and remaining half as jointing dressing. For all treatments, monocalcium phosphate and potassium chloride were applied as basal dose at 120 kg P2O5 ha−1 and 150 kg K2O ha−1, respectively. The experiment was a 2-way factorial arrangement of treatments within the randomized complete block design with 3 replications for each treatment and 4 m2 area for each plot. Other management followed the local standard practices in wheat production.

Exp. 2: different N rates and varieties. The experiment was undertaken on the experiment station of Jiangsu Academy of Agricultural Sciences located at Nanjing (118°52' E, 32°02' N) for a single season from 2003 to 2004. The soil of the field was classified as Gleyed paddy soil (Alfisols in U.S. taxonomy) with 9.6 g kg−1 organic matter, 1.0 g kg−1 total N, 40.29 mg kg−1 available phosphate, 102.78 mg kg−1 available potassium (0–25 cm soil depth). Four winter wheat cultivars as Huaimai 20, Xumai 26, Yangmai 10 and Ningmai 9 were planted on 2 Nov. 2003 with a density of 180 plants m−2. Urea N fertilizer was applied at 0, 75, 150, 225 and 300 kg N ha−1, N application was distributed as 60% for pre-planting basal, 20% for jointing dressing and 20% for booting dressing, respectively. For all treatments, monocalcium phosphate and potassium chloride were applied as basal dose at 150 kg P2O5 ha−1 and 112.5 kg K2O ha−1, respectively. The experiment was a 2-way factorial arrangement of treatments within the randomized complete block design with 3 replications for each treatment and 4 m2 area for each plot. Other management followed the local standard practices.

Exp. 3: different N rates and varieties. The experiment was undertaken on the experiment station of Jiangsu Academy of Agricultural Sciences located at Nanjing for a single season from 2004 to 2005. The soil of the field was classified as gleyed paddy soil (Alfisols in U.S. taxonomy) with 20.9 g kg−1 organic matter, 1.8 g kg−1 total N, 17.5 mg kg−1 available phosphate, 94.2 mg kg−1 available potassium. Three winter wheat cultivars, Ningmai 9, Yangmai 12, and Yumai 34, were planted on 2 Nov. 2004 with a density of 180 plants m−2. Urea N fertilizer was applied at 0, 90, 180 and 270 kg N ha−1, and the N application was distributed as 50% for pre-planting basal and 50% for jointing dressing. For all
treatments, monocalcium phosphate and potassium chloride were applied as basal dose at 80 kg P\(_2\)O\(_5\) ha\(^{-1}\) and 150 kg K\(_2\)O ha\(^{-1}\), respectively. The experiment was a 2-way factorial arrangement of treatments within the randomized complete block design with 3 replications for each treatment and 18 m\(^2\) for each plot. Other management followed local standard practices.

(2) The experiments with rice

Exp. 4: different N rates and water regimes. The experiment was conducted at Jiangpu experiment station of Nanjing Agricultural University (118°37'E, 32°01'N) in 2002 on Gleyed paddy soil (Alfisols in U.S. taxonomy). The soil contained 20.9 g kg\(^{-1}\) organic matter, 1.41 g kg\(^{-1}\) total N, 12.03 mg kg\(^{-1}\) available phosphate, 105.5 mg kg\(^{-1}\) available potassium (0–25 cm soil depth). Two japonica rice cultivars Nipponbare and Wuxiangjing 14 were planted on 16 May, and transplanted on 20 June with a density of 53.33 plants m\(^{-2}\). N fertilizer was applied at 0, 75, 150, 225, and 300 kg N ha\(^{-1}\). Urea N fertilizer was applied at 0, 105, 210, and 315 kg ha\(^{-1}\) as urea.

Exp. 6: different N rates and varieties. The experiment was undertaken at the Jiangning experiment station of Nanjing Agricultural Bureau (118°98'E, 31°94'N) in 2005. The field soil was classified as Gleyed paddy soil (Alfisols in U.S. taxonomy) with 16.1 g kg\(^{-1}\) organic matter, 1.36 g kg\(^{-1}\) total N, 10.36 mg kg\(^{-1}\) available phosphate, 82.6 mg kg\(^{-1}\) available potassium (0–25 cm soil depth). Two japonica rice cultivars Wuxiangjing 14 and 27123 were planted on 18 May, and transplanted on 20 June with a density of 53.33 plants m\(^{-2}\). N fertilizer was applied at 0, 90, 270, and 420 kg ha\(^{-1}\) as urea. N was applied in four splits (35% at pre-planting, 15% at tillering, 25% at jointing, and 25% at booting).

Exp. 7: different N rates and varieties. The experiment was undertaken at the Jiangning experiment station of Nanjing Agricultural Bureau (118°98'E, 31°94'N) in 2005. The field soil was classified as Gleyed paddy soil (Alfisols in U.S. taxonomy) with 16.1 g kg\(^{-1}\) organic matter, 1.36 g kg\(^{-1}\) total N, 10.36 mg kg\(^{-1}\) available phosphate, 82.6 mg kg\(^{-1}\) available potassium (0–25 cm soil depth). Two japonica rice cultivars Wuxiangjing 14 and 27123 were planted on 18 May, and transplanted on 20 June with a density of 53.33 plants m\(^{-2}\). N fertilizer was applied at 0, 90, 270, and 420 kg ha\(^{-1}\) as urea. N was applied in four splits (35% at pre-planting, 15% at tillering, 25% at jointing, and 25% at booting).

2. Measurements and data analysis

(1) Canopy spectral reflectance

In all the experiments, the spectral reflectance over the plant canopy of wheat and rice was periodically measured using a portable ground MSRI6 radiometer (Cropscan, Rochester, MN). Data were obtained on selected times to cover major growth stages from early jointing to physiological maturity under the suitable weather conditions. These individual times included 7 dates in Exp. 1 (15 and 30 March; 4, 12, 22 and 29 April; 16 May), 7 dates in Exp. 2 (8, 20 and 28 April; 4, 11, 17 and 24 May), 5 dates in Exp. 3 (13 and 26 April; 6, 18 and 25 May), 5 dates in Exp. 4 (1, 20 and 30 Aug.; 13 and 26 Sep.), 6 dates in Exp. 5 (5, 12, 22, and 26 Sep.; 8 and 15 Oct.), 7 dates in Exp. 6 (19 and 27 Aug.; 2, 11, 23 and 30 Sep.; 12 Oct.), and 5 dates in Exp. 7 (9 and 16 Aug.; 5, 13 and 29 Sep.), respectively. The wavelength range of 447-1752 nm carried 16 specific wavebands centered at 460, 510, 560, 610, 660, 680, 710, 760, 810, 870, 950, 1100, 1220, 1480, 1500, 1650 nm. A data acquisition device (Cropscan Inc, 2000) equipped with sun angle cosine correction capacity was used to record reflectance data.

During the experiments, periodical measurements were made at five sites over each plot, looking straight down from more than 1.0 m above the crop canopy, and with a 31.1° field of view. Radiometer calibration was conducted daily with an opal glass diffuser using the two-point (2 Point Up/Down) method (DLC Model 2000, Cropscan Inc.). All spectral measurements were made on cloudless or near cloudless days between 1100–1400 h.

(2) Leaf nitrogen concentration (LNC)

After each measurement of canopy spectral reflectance, 5 to 10 plants were randomly selected from each plot and harvested for determination of leaf N concentration. From each sample, all green
leaves were separated from the stems, and oven-dried at 70°C to constant weight, then weighed. The dried leaf samples were ground to pass 1-mm screen, and then stored in plastic bags for chemical analysis. Total N concentration in leaf tissues was determined by the micro-Kjeldahl method and LNC (mg N·g⁻¹ DW) was expressed on the basis of unit dry weight.

(3) Calculations and statistical analysis
The data from Exp. 1 and 2, and Exp. 4, 5 and 6 were used for deriving equations to predict LNC in wheat and rice, respectively, and the data from Exp. 3 and 7 were used for testing the derived equations in wheat and rice, respectively.

The effects of N treatments on LNC were analyzed statistically by comparing the means of each treatment using Duncan’s multiple comparison at a probability less than 0.05 (SAS Institute, 2004). Since the observations from the different cultivars and growth stages in wheat and rice essentially showed similar patterns in response to varied N rates, the experimental data from wheat (156 samples) or rice (105 samples) crops were pooled separately for comprehensive inspection of dynamic change patterns of LNC and canopy spectral reflectance under different experiment treatments. First, linear and nonlinear (Spearman’s correlation coefficient) correlation analyses were conducted between the spectral reflectance of all 16 bands and LNC so that sensitive spectral ranges (known as key bands) related to LNC were identified (SAS Institute, 2004). Second, using a self-developed computer program based on the software of MATLAB 6.0 (The MathWorks, Inc., Natick, MA), different spectral parameters (Table 1) comprised of the key bands were calculated from the raw reflectance data, and linear or nonlinear correlation analyses were conducted between the spectral parameters and LNC so that sensitive spectral parameters related to LNC were identified. Then, separate linear or nonlinear models were fitted to determine the best-fit $R^2$ values for the relationships of LNC to the canopy spectral reflectance of key bands and spectral parameters with SAS software (SAS Institute, 2004). When no linearity existed, exponential, power, logarithmic or quadratic models were tested. Finally, five relationships with best-fit $R^2$ values in wheat or rice were selected separately, from which common wavebands and spectral parameters were explored for indicating LNC in wheat and rice, and monitoring equations were further formulated.

The monitoring equations with common spectral parameters were tested with the data from Exp.3 and 7 for wheat and rice, respectively. In equation testing, the predicted results were compared with the field measurements to evaluate reliability and accuracy of the equation output under practical conditions. The following statistic method, root mean square error (RMSE, % value) against the observed mean, was used to calculate the fitness between the estimated results and observed data (Loague and Green, 1991):

$$\text{RMSE} = \left( \frac{\sum_{i=1}^{n} (P_i - O_i)^2}{n} \right)^{1/2} \times \frac{100}{\bar{O}}$$

where $P_i$ and $O_i$ are predicted and observed values, respectively; $\bar{O}$ is the observed mean value. RMSE (%) shows the relative difference between the predicted and observed data. The prediction is considered excellent with the RMSE <10%, good if 10–20%, fair if 20–30%, poor if >30% (Jamieson et al., 1991).

Results

1. Leaf nitrogen concentration of wheat and rice under different N rates
The LNC is a major indicator to characterize the leaf N status in rice and wheat. The data in Tables 2 and 3 summarized the LNC at four main growth stages of different cultivars under varied N rates in experiments 1, 2, 4, 5 and 6 to display the response patterns of leaf N status. The results show that the LNC at individual growth stages of all cultivars in either wheat or rice exhibited differences with varied N fertilization rates, tending to rise with increasing N rates, with slight difference between the higher N rates. With wheat, the range of the LNC between the lowest and highest N rates of 0 to 300 kg N·ha⁻¹ varied from 5.2 mg N·g⁻¹ DW to 47.2 mg N·g⁻¹ DW among different growth stages and cultivars. In rice, the range of the LNC between the lowest and highest N rates of 0 to 315 kg N·ha⁻¹ varied from 11.6 mg N·g⁻¹ DW to 36.7 mg N·g⁻¹ DW at different growth stages and cultivars. In wheat, there were slight differences among different cultivar types at the stages of jointing and booting in each year, with greater LNC

| Spectral parameter | Abbreviation | Algorithm formula |
|--------------------|--------------|------------------|
| Reflectance        | $\rho_\lambda$ |                  |
| Ratio vegetation index | RVI ($\lambda_1, \lambda_2$) | $\rho_{\lambda_1}/\rho_{\lambda_2}$ |
| Differential vegetation index | DVI ($\lambda_1, \lambda_2$) | $\rho_{\lambda_1}-\rho_{\lambda_2}$ |
| Normalized difference vegetation index | NDVI ($\lambda_1, \lambda_2$) | $(\rho_{\lambda_1}-\rho_{\lambda_2})/(\rho_{\lambda_1}+\rho_{\lambda_2})$ |

$\lambda$ is the wavelength.

Table 1. Algorithm of different spectral parameters used in the paper.

---

Zhu et al. —— Monitoring Leaf Nitrogen in Wheat and Rice 403
values in high grain protein cultivars (e.g. Xumai 26) than in lower grain protein cultivars (e.g. Huaimai 18). Yet by anthesis and filling stages there were no clear differences in the pattern of LNC among cultivars, probably caused by different N remobilization strengths from leaves to grains after anthesis in these cultivars. Furthermore, the LNC values in wheat in 2001−2002 were greater than the measurements in 2003−2004 under similar N application rates, because slight soil waterlogging occurred during the periods of the field experiment in 2004, which could have affected N uptake and utilization by wheat plants. In rice, although there was no obvious difference among different cultivars, the LNC values in 2003 were greater than the measurements in 2002 under similar N application rates. This may be because in 2003 higher precipitation and less radiation occurred over the growing period of rice crop, which resulted in relatively lower dry matter accumulation and leaf area index, thus relatively higher LNC. In addition, there were slight differences between two water regimes in rice, with a little greater LNC values in shallow water system than in intermittent irrigation system.

The overall results on LNC indicated that the varied N fertilization rates in both rice and wheat affected leaf N status, which in turn affected radiation absorption and reflection by the crop canopy under different N treatments. The treatment differences were enhanced by the different cultivars and years and created a wide range of variation in leaf N status and canopy spectral reflectance so that the quantitative relationships found should be applicable to diverse N nutrition levels.

Table 2. Leaf nitrogen concentration (LNC) at jointing, booting, anthesis, and filling stages of different wheat cultivars under different N levels in two years.

| Year          | N rate (kg N·ha⁻¹) | Cultivar   | LNC (mg N·g⁻¹DW) |
|---------------|---------------------|------------|------------------|
|               |                     |            | Jointing | Booting | Anthesis | Filling |
| 2001−2002     | 0                   | Xumai 26   | 38.5b     | 33.9c   | 25.6b    | 23.8b   |
|               | 120                 |            | 44.3a     | 41.6b   | 34.9a    | 31.8a   |
|               | 210                 |            | 46.9a     | 44.1a   | 35.7a    | 33.8a   |
|               | 300                 |            | 46.3a     | 44.6a   | 35.2a    | 33.8a   |
|               | 0                   | Huaimai 18 | 37.2b     | 32.5b   | 25.1c    | 27.1c   |
|               | 120                 |            | 43.8a     | 39.9a   | 30.9b    | 30.3b   |
|               | 210                 |            | 44.3a     | 41.0a   | 32.8a    | 34.2a   |
|               | 300                 |            | 46.3a     | 42.0a   | 36.2a    | 34.1a   |
| 2003−2004     | 0                   | Huaimai 20 | 20.0b     | 22.1c   | 20.5c    | 17.8d   |
|               | 250                 |            | 20.7b     | 21.9c   | 21.1c    | 20.3c   |
|               | 300                 |            | 22.4b     | 22.7c   | 23.7b    | 23.5c   |
|               | 0                   | Ningmai 9  | 26.0ab    | 26.9b   | 27.4ab   | 27.6ab  |
|               | 150                 |            | 22.9ab    | 23.1b   | 25.1ab   | 22.3c   |
|               | 225                 |            | 24.9ab    | 26.9a   | 25.8a    | 29.2b   |
|               | 300                 |            | 27.1a     | 29.5a   | 26.6a    | 30.9a   |
|               | 0                   | Xumai 26   |           |         | 17.9d    | 16.6c   |
|               | 75                  |            |           |         | 21.9c    | 20.3b   |
|               | 150                 |            |           |         | 23.9c    | 21.3b   |
|               | 225                 |            |           |         | 27.4b    | 25.6a   |
|               | 300                 |            |           |         | 30.5a    | 28.5a   |
|               | 0                   | Yangmai 10 |           |         | 20.0c    | 18.4d   |
|               | 75                  |            |           |         | 21.7c    | 22.0c   |
|               | 150                 |            |           |         | 22.6c    | 22.7c   |
|               | 225                 |            |           |         | 27.4b    | 25.7b   |
|               | 300                 |            |           |         | 33.0a    | 28.9a   |

†Values per cultivar in each column followed by different letters indicate significant difference at 0.05 probability level.
2. Canopy reflectance spectra under different N rates

The reflectance spectra of canopy at different growth stages were markedly different under varied N rates, with consistent patterns evident among different cultivars in rice and wheat. The canopy spectral reflectance under five N rates in wheat of Huaimai 18 cultivar at anthesis in Exp. 2 (Fig. 1A) and in rice of Wuxiangjing 9 cultivar at full heading in Exp. 5 (Fig. 1B) were taken as examples to display the response patterns of canopy reflectance spectra to varied N rates, respectively. With both crops, in the visible wavebands (460−710 nm) and near infrared (NIR) long wavebands (1480−1650 nm), the spectral reflectance decreased with rising N rates, whereas in the NIR short wavebands (760−1220 nm) the reflectance tended to increase with increasing N rates, resulting in the greater differences among the N rates in NIR short wavebands than in visible wavebands. Overall, the variation range of the spectral reflectance under different N rates was smaller in rice than in wheat, especially in the visible wavebands (460−710 nm) and NIR long wavebands (1480−1650 nm), probably due to smaller variation range of LNCs in rice. The canopy spectral reflectance of rice was lower than that of wheat under the same N rate, which could be resulted from water layer in the wet rice. In addition, greater leaf area index in rice than in wheat is another reason for lower canopy spectral reflectance in the visible wavebands in rice than in wheat. These dynamic patterns of canopy reflectance under varied N rates with different wheat and rice cultivars provided basis for the analysis of common sensitive wavebands and

Table 3. Leaf nitrogen concentration (LNC) at initial heading, full heading, initial filling, and full filling stages of different rice cultivars under different N levels in three years

| Year | N rate (kg N·ha⁻¹) | Cultivar | LNC (mgN·g⁻¹DW) |
|------|------------------|----------|-----------------|
|      |                  |          | Initial heading | Full heading | Initial filling | Full filling |
| 2002 | 0                | Wuxiangjing 9 (Shallow water system) | 21.2d | 17.9d | 15.9b |
|      | 75               | Wuxiangjing 9 (Shallow water system) | 24.0c | 20.5c | 16.6b |
|      | 150              | Wuxiangjing 9 (Shallow water system) | 26.1bc | 25.1b | 18.4ab |
|      | 225              | Wuxiangjing 9 (Shallow water system) | 27.0b | 25.8ab | 22.3a |
|      | 300              | Wuxiangjing 9 (Shallow water system) | 29.7a | 27.3a | 22.6a |
|      | 0                | Wuxiangjing 9 (Intermittent irrigation system) | 19.8c | 19.4c | 12.5c |
|      | 75               | Wuxiangjing 9 (Intermittent irrigation system) | 23.4b | 21.1bc | 15.0bc |
|      | 150              | Wuxiangjing 9 (Intermittent irrigation system) | 25.3ab | 22.6b | 13.3c |
|      | 225              | Wuxiangjing 9 (Intermittent irrigation system) | 27.9a | 23.3b | 19.3ab |
|      | 300              | Wuxiangjing 9 (Intermittent irrigation system) | 27.9a | 26.9a | 22.4a |
| 2003 | 0                | Wuxiangjing 9 (Shallow water system) | 25.7d | 19.3b | 14.3c |
|      | 75               | Wuxiangjing 9 (Shallow water system) | 26.7c | 23.4ab | 19.9c |
|      | 150              | Wuxiangjing 9 (Shallow water system) | 27.1c | 25.6a | 19.0b |
|      | 225              | Wuxiangjing 9 (Shallow water system) | 29.0b | 26.1a | 21.8ab |
|      | 300              | Wuxiangjing 9 (Shallow water system) | 30.2a | 26.7a | 24.4a |
|      | 0                | Wuxiangjing 9 (Intermittent irrigation system) | 22.6c | 17.3b | 15.3a |
|      | 75               | Wuxiangjing 9 (Intermittent irrigation system) | 25.9b | 19.6ab | 16.4a |
|      | 150              | Wuxiangjing 9 (Intermittent irrigation system) | 28.1ab | 24.9ab | 18.8a |
|      | 225              | Wuxiangjing 9 (Intermittent irrigation system) | 28.1ab | 25.1ab | 19.4a |
|      | 300              | Wuxiangjing 9 (Intermittent irrigation system) | 29.3a | 26.0a | 21.4a |
| 2004 | 0                | Nipponbare | 23.1b | 18.9a | 17.5a |
|      | 105              | Nipponbare | 25.2a | 19.6a | 18.0a |
|      | 210              | Nipponbare | 26.5a | 19.7a | 18.2a |
|      | 315              | Nipponbare | 27.1a | 22.8a | 18.7a |
|      | 0                | Huajing 2 | 20.9c | 24.8a | 19.9a | 14.5b |
|      | 105              | Huajing 2 | 26.3b | 26.2a | 20.3a | 15.8ab |
|      | 210              | Huajing 2 | 28.2a | 27.7a | 22.9a | 16.9ab |
|      | 315              | Huajing 2 | 28.3a | 29.0a | 22.4a | 17.5a |

*Values per cultivar in each column followed by different letters indicate significant difference at 0.05 probability level.*
spectral parameters and deriving quantitative relationships of leaf N status to spectral characters of canopy reflectance.

### 3. Relationship of LNC to canopy spectral reflectance

Linear and nonlinear correlation analyses were conducted between the canopy spectral reflectance of all 16 wavebands and LNC from pooled data of 156 pairs obtained at different growth stages under the different treatments of two wheat experiments (Exp. 1 and 2). The linear correlation analyses indicated that in general the LNC in wheat was negatively related to the reflectance in visible (460–710 nm) and NIR long wavebands (1480–1650 nm), and positively related to NIR short wavebands (710–1220 nm) with 0.01 significant levels, as seen in Table 4. The analyses of nonlinear correlation also showed that the LNC in wheat was related to the reflectance of all 16 wavebands with 0.01 significant levels. Integrating these results of linear and nonlinear correlations, the 610, 660 and 680 nm had the highest correlation in wheat. Table 5 listed 5 different wavebands and regression equations with highest correlations in wheat. Among them, the coefficients of determination ($R^2$, 0.80–0.87) were very close against the wavebands of 610, 660, 680, 510 and 760 nm.

![Canopy reflectance under different N rates](image)

**Fig. 1.** Canopy reflectance under different N rates in wheat of Huaimai 20 at anthesis in Exp.2 (A) and in rice of Wuxiangjing 9 at full heading in Exp.5 (B).

| Wavelength (nm) | Rice   | Wheat | Wavelength (nm) | Rice   | Wheat |
|-----------------|--------|-------|-----------------|--------|-------|
|                 | Linear | Nonlinear | Linear | Nonlinear | Linear | Nonlinear | Linear | Nonlinear | Linear | Nonlinear |
| 460             | -0.03  | 0.05   | -0.78**        | -0.84** |       | 810     | 0.36** | 0.35**   | 0.89** | 0.92**   |
| 510             | -0.70**| -0.67**| -0.85**        | -0.92** |       | 870     | 0.31** | 0.30**   | 0.88** | 0.91**   |
| 560             | -0.71**| -0.75**| -0.87**        | -0.91** |       | 950     | 0.08   | 0.20*    | 0.86** | 0.90**   |
| 610             | -0.82**| -0.84**| -0.88**        | -0.94** |       | 1100    | 0.08   | 0.17     | 0.85** | 0.89**   |
| 660             | -0.84**| -0.81**| -0.86**        | -0.95** |       | 1220    | 0.14   | 0.19     | 0.76** | 0.79**   |
| 680             | -0.84**| -0.84**| -0.85**        | -0.94** |       | 1480    | -0.14  | -0.15    | -0.49** | -0.42**  |
| 710             | -0.42**| -0.72**| -0.85**        | -0.89** |       | 1500    | -0.38**| -0.44**  | -0.63** | -0.56**  |
| 760             | -0.24* | 0.27** | 0.80**         | 0.91**  |       | 1650    | -0.22**| -0.24**  | -0.62** | -0.56**  |

*, **, significant at $P<0.05$, $P<0.01$, respectively.
4. LNC in rice was positively related to NIR short wavebands (760–1220 nm), but with 0.01 significant level only at 810 nm and 870 nm, and with 0.05 significant level at 760 nm. LNC in rice was negatively related to NIR long wavebands (1480–1650 nm) with 0.05 significant level only at 1650 nm, and with 0.01 significant level only at 1500 nm. In addition, the analyses of nonlinear correlation showed that LNC in rice was related to the reflectance at 510−870 nm and 1500 nm with 0.01 significant level, and at 950 nm and 1650 nm with 0.05 significant level. In both linear and nonlinear correlation analyses, the 610, 660 and 680 nm had the highest correlation in rice. Table 5 listed 5 different bands and regression equations with highest correlations in rice. It can be seen that the $R^2$ values for the best correlation in rice between reflectance of single waveband and LNC were significantly lower than in wheat. Yet following a similar pattern as in wheat, the $R^2$ values at the bands of 610, 660 and 680 nm for rice were close to each other (0.70–0.75), whereas the $R^2$ decreased markedly at the bands of 560 and 710 nm. Thus, the 610, 660 and 680 nm can be considered as three common wavebands with high correlation to LNC in both rice and wheat.

![Fig. 2. Relationships between canopy reflectance at 680 nm (A) or 610 nm (B) and LNC in wheat and rice.](image)

| Crop type | Wavelength (nm) | Regression equation | $R^2$ |
|-----------|-----------------|---------------------|-------|
| Wheat (n=156) | 680      | $y=70.0x^{0.99}$     | 0.87  |
| Wheat (n=156) | 660      | $y=68.9x^{0.99}$     | 0.88  |
| Wheat (n=156) | 610      | $y=116.9x^{1.12}$    | 0.85  |
| Wheat (n=156) | 510      | $y=99.3x^{1.43}$     | 0.83  |
| Wheat (n=156) | 760      | $y=32.7Ln(x)−84.9$   | 0.80  |
| Rice (n=105)  | 680      | $y=37.5x^{0.40}$     | 0.75  |
| Rice (n=105)  | 660      | $y=38.0x^{0.40}$     | 0.72  |
| Rice (n=105)  | 610      | $y=46.5x^{0.53}$     | 0.70  |
| Rice (n=105)  | 560      | $y=−11.9Ln(x)+40.7$  | 0.52  |
| Rice (n=105)  | 710      | $y=−11.2Ln(x)+45.9$  | 0.52  |

4. Relationship of LNC to canopy spectral parameters

All possible RVIs, DVIs and NDVIs were calculated...
from the above three common wavebands (610, 660 and 680 nm) and 16 individual wavebands. The correlations between the LNC of wheat and all possible RVI, DVIs and NDVIs were analyzed with the combined data of two wheat experiments (Exp.1 and 2). Table 6 listed 5 spectral parameters and regression equations with highest correlations in wheat. It can be seen that the LNC relation with 5 best spectral parameters gave much greater $R^2 (>0.91)$ than the relation to the reflectance of 5 best single wavebands ($R^2 > 0.80$). This is mainly because the spectral parameters derived from the sensitive wavebands could minimize the influence of soil and measurement backgrounds and thus enhanced the information of the sensitive wavebands, leading to more steady and reliable monitoring of leaf N status in crop plants (Huete et al., 1985). Among different relationships developed to relate LNC of wheat to spectral parameters, that developed with NDVI (1220, 610) had the best fit ($R^2$ over 0.92 with a sample number of 156, Table 6, Fig. 3A).

Similarly, the relationships between the LNC of rice and all possible RVIs, DVIs and NDVIs were analyzed with the combined data of three rice experiments (Exp. 4, 5 and 6). Table 6 listed 5 spectral parameters and regression equations with highest correlations in rice. As compared to 5 single wavebands with best correlation, the $R^2 (>0.75)$ of 5 spectral parameters with highest correlation in rice is greater than that of 5 single wavebands ($R^2 > 0.52$). Similar with wheat, NDVI (1220, 610) had the best fit ($R^2$ over 0.78 with a sample number of 105, Table 6, Fig. 3A) among all spectral parameters in rice. In addition, Table 6 showed that the RVI(1220, 610) was also closely linked to the LNC in both rice and wheat, thus NDVI (1220, 610) and RVI(1220, 610) can be considered as two common

---

**Table 6. Quantitative relationships of LNC (y) to canopy spectral parameter (x) of 5 best in wheat and rice.**

| Crop type | Canopy spectral parameter | Regression equation | $R^2$ |
|-----------|---------------------------|---------------------|-------|
| Wheat (n=156) | NDVI(1220, 610) | $y = 47.3x^{1.63}$ | 0.92 |
| | RVI(1220, 660) | $y = 13.8Ln(x) - 1.2$ | 0.92 |
| | RVI(1220, 610) | $y = 16.5Ln(x) - 2.9$ | 0.91 |
| | RVI(810, 660) | $y = 11.3Ln(x) + 0.074$ | 0.91 |
| | NDVI(1220, 710) | $y = 56.3x^{1.05}$ | 0.91 |
| Rice (n=105) | NDVI(1220, 610) | $y = 5.8e^{2.0x}$ | 0.78 |
| | RVI(1220, 610) | $y = 11.2Ln(x) + 3.6$ | 0.77 |
| | NDVI(1220, 680) | $y = 5.8e^{3.8x}$ | 0.76 |
| | RVI(1220, 680) | $y = 9.3x^{0.44}$ | 0.76 |
| | NDVI(810, 610) | $y = 5.0e^{1.97x}$ | 0.75 |

---

Fig. 3. Relationships between NDVI (1220, 610) (A) or RVI (1220, 610) (B) and LNC in wheat and rice.
spectral parameters indicating LNC in both rice and wheat. Fig. 3 displays the relationships of LNC to the NDVI(1220, 610) and RVI(1220, 610) in rice and wheat. Analysis of covariance indicated that the change patterns of LNC to the NDVI(1220, 610) and RVI(1220, 610) were different between crop species with the significance difference at 0.05 probability level, and thus different regression coefficients were better used for precisely estimating the LNC of different crops. Compared with single sensitive wavebands (680, 660 and 610 nm), the NDVI(1220, 610) and RVI(1220, 610) had the higher $R^2$ for their functions, thus the NDVI(1220, 610) or RVI(1220, 610) with different regression coefficients could be used for estimating LNC in rice and wheat.

5. Testing of LNC relationships to canopy reflectance spectra

The relationships of LNC to NDVI(1220, 610) or RVI(1220, 610) were tested using the data from Exp. 3 that included three different wheat cultivars and four different N application rates in the single season of 2004 to 2005 and from Exp. 7 that included two different rice cultivars and four different N application rates in 2005. Comparison of the observed with the estimated LNC using NDVI(1220, 610) and RVI(1220, 610) had the higher R$^2$ for their functions, thus the NDVI(1220, 610) or RVI(1220, 610) with different regression coefficients could be used for estimating LNC in rice and wheat.

Fig. 4. Comparison between observed and predicted LNC with NDVI (1220, 610) (A) and RVI (1220, 610) (B) in three wheat cultivars under four N rates in Exp. 3 in 2004–2005.

Discussion

Through comprehensive analyses on the field experiment data in wheat and rice involving different N rates, cultivar types and growth stages in different years, the present study indicated that 610, 660 and 680 nm could be considered as three common sensitive wavebands of LNC in both wheat and rice, and the LNC in both wheat and rice could be monitored with common spectral parameters [NDVI(1220, 610) and RVI(1220, 610)] of canopy reflectance, while different regression coefficients were better used for precisely estimating the LNC of different crops. Testing of the regression equations in rice and wheat with independent dataset indicated a good fit between the estimated and observed LNC. These results have provided a technical approach for non-destructive monitoring and diagnosis of leaf N status in cereal crops, based on plant canopy reflectance from remote sensing. This information should be useful for development of portable leaf N meter with ground-based spectral reflectance and for extraction of spectral parameters from air-borne images, which is particularly applicable to the regions with rice-wheat
rotation as a major double cropping system, such as in central and east China.

As compared to the previous studies with single crops, the 660 nm for LNC in both wheat and rice were within the range of the 630−660 nm proposed by Wang et al. (1993) for N concentration of rice, and 680 nm for LNC in both wheat and rice were within the sensitive wavebands of 430, 550 and 680 nm reported by Filella et al. (1995), and the 660, 680 and 610 nm for LNC in both wheat and rice are all within the range of 550−710 nm reported by Blackmer et al. (1996). The NDVI(1220, 610) or RVI(1220, 610) for LNC in both wheat and rice were different from the combination of two spectral wavebands of 671 and 780 nm for LNC in wheat proposed by Stone et al. (1996), and RVI(660, 460) and NDVI(660, 460) for LNC in wheat reported by Xue et al. (2004), and different from the reflectance factors at 620 nm and 760 nm (regression with 2 variables) or 400 nm, 620 nm and 880 nm (regression with 3 variables) for LNC in rice reported by Shibayama and Akiyama (1986). Thus, this research has exhibited new choices of key wavebands and spectral parameters for non-destructive monitoring of leaf N status in both rice and wheat crops under varied N levels.

Testing of the regression equations with NDVI(1220, 610) or RVI(1220, 610) in rice and wheat indicated that these equations could be used for a reliable estimation of the LNC in both wheat and rice, but the estimation accuracy appeared to be better for wheat than for rice in this research. From authors’ experience in the past 5 years, the differential accuracy could have resulted from greater observation errors in the experimentation of rice than of wheat. Firstly, the measurements on canopy spectral reflectance was affected more by background reflection from flooded or wet paddy fields than from relatively dry and uniform wheat fields. Secondly, during grain filling period, the color loss on panicles was faster than on leaves in rice, this non-synchronous greenness pattern within canopy would have an impact on actual canopy spectral reflectance in rice, whereas in wheat the color loss on spikes and leaves was more consistent than in rice. Thirdly, after stem elongation, there were more obvious variation or less uniform growth among individual plant stems per hill in rice as compared to single seeded wheat plant, this would increase sampling error of individual stems for tissue N assay in rice. Since there is a lack of literature on similar research using both rice and wheat, more careful study is needed to further elucidate the reasons for different monitoring accuracy among rice and wheat so that measures could be taken to make non-destructive monitoring of N status in rice leaves more reliable.

It should be noted that the present results were obtained from one ecological region, although several experiments were conducted at different sites near Nanjing of China. This implies that the above monitoring equations for indicating LNC in rice and wheat should be further tested with independent datasets from diverse ecological areas and production systems, which will help to make them more reliable and useful under a range of conditions. In addition, improved statistical models (Takahashi et al., 2000)
may be used for enhancing accuracy in prediction of LNC in crops, and the regression coefficients should be carefully evaluated for different monitoring equations. These issues would be taken into consideration in future research with rice and wheat.

Acknowledgement

We acknowledge the financial support of the National Natural Science Foundation of China (30400278, 30571092, 30671215), State Hi-tech R&D Plan of China (2006AA10A303), Natural Science Foundation of Jiangsu Province (BK2005079, BK2005212), and PhD Program Grant of China (20060307031) for this research.

References

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

Cropscan Inc. 2000. Data logger controller, User’s guide and technical reference. CROPSCAN Inc. Rochester, MN.1-292.

Curran, P.J., Dungan, J.L. and Peterson, D.L. 2001. Estimating the foliar biochemical concentration of leaves with reflectance spectrometry : testing the Kokaly and Clark methodologies. Remote Sens. Environ. 76 : 349-359.

Everitt, J.H., Petitt, R.D. and Alaniz, M.A. 1987. Remote sensing of broom snake weed ( Gutierrezia sarothrae ) and spiny aster ( Aster spinosus ). Weed Sci. 35 : 295-302.

Filella, I., Serrano, L., Serra, J. and Penuelas, J. 1995. Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. Crop Sci. 35 : 1400-1405.

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

Jamieson, P.D., Porter, J.R. and Wilson, D.R. 1991. A test of the computer simulation model ARC-WHEAT1 on wheat crops grown in New Zealand. Field Crops Res. 27 : 337-350.

Janssen, A. and Lorenzen, B. 1990. Radiometric estimation of biomass and nitrogen content of barley grown at different nitrogen levels. Int. J. Remote Sens. 11 : 1809-1820.

Johnkutty, I., Mathew, G., Thiayagarajan, T.M. and Balasubramanian, V. 2000. Relationship among leaf nitrogen content, SPAD and LCC values in rice. J. Trop. Agric. 38 : 97-99.

Jongschaap, R.E.E. and Boojj, R. 2004. Spectral measurements at different spatial scales in potato : relating leaf, plant and canopy nitrogen status. Int. J. Applied Earth Obs. Geoinf. 5 : 295-218.

Lee, T., Raja, K. and Reddy, G. 2000. Reflectance indices with precision and accuracy in predicting cotton leaf nitrogen concentration. Crop Sci. 40 : 1814-1819.

Li, J.H., Dong, Z.X. and Zhu, J.Z. 2003. Present application and outlook for method of nitrogen nutrition diagnosis. J. Shihwei Univ. (Natural Sci.) 7 : 80-83*.

Liu, L., Sang, D., Liu, C., Wang, Z., Yang, J. and Zhu, Q. 2003. Effects of real-time and site-specific nitrogen managements on rice yield and nitrogen use efficiency. Scientia Agric. Sinica. 36 : 1456-1461.

Loague, K. and Green, R.E. 1991. Statistical and graphical methods for evaluating solute transport models : overview and application. J. Contam. Hydrol. 7 : 51-73.

Niamatungiro, S., Norman, R.J., McNew, R.W. and Wells, B.R. 1999. Comparison of plant measurements for estimating nitrogen accumulation and grain yield by flooded rice. Agron. J. 91 : 676-685.

Raun, W.R. and Johnson, G.V. 1999. Improving nitrogen use efficiency for cereal production. Agron. J. 91 : 357-363.

Roth, G.W. and Fox, R.H. 1989. Plant tissue test for predicting nitrogen fertilizer requirement of winter wheat. Agron. J. 81 : 502-507.

SAS Institute. 2004. SAS/STAT user’s guide. Version 9.1. SAS Inst., Cary, NC. 25-68.

Shibayama, M. and Akiyama, T.A. 1986. A spectroradiometer for field use. VII. Radiometric estimation of nitrogen levels in field rice canopies. Jpn. J. Crop Sci. 55 : 439-445.

Stone, M.L., Soile, J.B., Raun, W.R., Whitney, R.W., Taylor, S.L. and Ringer, J.D. 1996. Use of spectral radiance for correcting in-season fertilizer nitrogen deficiencies in winter wheat. Trans. ASAE 39 : 1629-1631.

Takahashi, W., Nguyen-Cong, V., Kawaguchi, S., Minamiyama, M. and Ninomiya, S. 2000. Statistical models for prediction of dry weight and nitrogen accumulation based on visible and near-infrared hyper-spectral reflectance of rice canopies. Plant Prod. Sci. 3 : 377-386.

Thenkabail, P.S., Smith, R.B. and Pauw, E.D. 2000. Hyper-spectral vegetation indices and their relationships with agricultural crop characteristics. Remote Sens. Environ. 71 : 158-182.

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

Wang, R.C., Chen, M.Z. and Jiang, H.X. 1993. Studies on agronomic mechanism of the rice yield estimation by remote sensing : I. The rice reflectance characteristics of different nitrogen levels and the selection of their sensitive bands. J. Zhejiang Agric. Univ. 19 (suppl.) : 7-14*.

Xue, L.H., Cao, W.X., Luo, W.H. and Zhang, X. 2004. Correlation between leaf nitrogen status and canopy spectral characteristics in wheat. Acta Phytoecologica Sinica. 28 : 172-177*.

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

Zhang, F. and Ma, W. 2000. The relationship between fertilizer input level and nutrient use efficiency. Soil and Environ. Sci. 9 : 154-157*.

* In Chinese with English abstract