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Nitrogen Contents of Rice Panicle and Paddy by Hyperspectral Remote Sensing

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Abstract: The nitrogen content or crude protein content in rice grains is one of the important indices to evaluate the nutrition and taste quality of rice. Normal determination of their contents by chemical methods is highly expensive and time consuming. The hyperspectral reflectances of the canopy, flag leaf and panicle of 5 rice varieties are measured by a ASD FieldSpec Pro FR™ in field under 3 nitrogen support levels in maturing process. The nitrogen contents of stems, leaves, flag leaves, panicles and rice paddy and their crude protein contents are determined. The correlation among them is analyzed. The panicles nitrogen contents (%) are very significantly correlate not only to that of stems, leaves and flag leaves and chlorophyll contents (mg g^{-1}) of flag leaves at milking and maturing stages, but also to the spectral reflectance ρ_λ, the first derivative spectra D_λ and RVI of canopy, flag leaf and panicle itself. The nitrogen contents (%) of rice paddy are very significantly correlative to that of stems and leaves and the spectral reflectance ρ_λ, the first derivative spectra D_λ and RVI of canopy at some wave bands at booting, milking and maturing stages. For the squared multiple correlation coefficients (R^2) of estimating the nitrogen contents of panicle and paddy by canopies spectra, we find R^2>0.80 at milking stage, R^2>0.75 at maturing stage, but for the estimation of panicle by the spectra of flag leaf and panicle itself, we have R^2>0.65. It indicates that it can be feasible for estimating the contents of nitrogen and crude protein in rice grains by hyperspectral remote sensing. It provide basis for monitoring rice quality by remote sensing.

Key words: Nitrogen content, rice panicle, rice paddy, flag leaf, reflectance, hyperspectral remote sensing

INTRODUCTION

The relations between agronomic parameters, such as Leaf Area Index (LAI), above ground biomass, fresh leaf mass, the contents of chlorophyll and carotenoid and nitrogen content of crops and their reflective spectra have been studied by monitoring their spectral changes in different growing stages (Shibayama and Akiyama, 1989; Blackburn, 1998; Wang *et al*., 1998; Tang *et al*., 2004). It provides a basis of growth monitoring and yields estimation for crops by remote sensing (Xu and Wang, 2001). The reflective spectra of crop canopies and leaves and their variables have been estimated their agronomic parameters, in particular pigment and nitrogen contents (Card *et al*., 1988; Wang *et al*., 1998), because they are mostly influenced by vegetation pigment (chlorophyll and carotenoid) and covering rate in the visible region and by the structure of canopy and leaf, above ground biomass, biochemical components such as protein, cellulose and starch in the near infrared region (Gausman *et al*., 1970). In recent years, the hyperspectral remote sensing has a mighty advantages on the vegetation remote sensing and its application because it could be used to quantitatively analyze a feebleness spectral difference (Vane, 1993). It is important for growth monitoring and yields estimation for rice that its spectral reflectance is used to estimate the chlorophyll content and nitrogen status (Takebe *et al*., 1990; Shibayama and Akiyama, 1991). Former tests have indicated that it is feasible for estimating the contents of leaf nitrogen or its crude protein from the canopy spectra by hyperspectyral remote sensing (Card *et al*., 1988; Niu *et al*., 2002). The contents of protein, cellulose and lignin of dry leaf are better to be estimated by their hyperspectra (Kokaly and Clark, 1999; Kokaly, 2001; Curran *et al*., 2001). There are some reports on estimating LAI, above ground biomass, yields, the contents of nitrogen, cellulose and lignin for rice, but very few for the contents of nitrogen, crude protein and crude starch of panicle and paddy on studying rice by hyperspectra. The nitrogen content or crude protein content is one of the important indices evaluating rice nutritious quality and one of the factors influencing on rice cooking quality (Martin and Fitzgerald, 2002).

In present study, hyperspectral reflectances of the canopy, flag leaf and panicle of rice are measured by an ASD FieldSpec Pro FR™ in field and indoor. The contents

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of chlorophyll and nitrogen of leaf, panicle and paddy corresponding to the spectra are determined by the biochemical method. The correlation between the nitrogen contents of grains (panicle, paddy) and their hyperspectra, the canopy spectra are analyzed. This study can provide some basis of quality monitoring of rice by hyperspectral remote sensing.

MATERIALS AND METHODS

Experiment design

Plant material: Rice (*Oryza sativa* L.) cv. Xiushui 110 (common *Japonica* rice with about 145 d of whole growing period, erect and compact), Xieyou 9308 (hybrid *Indica* rice with about 140 days of whole growing period, erect from disheveled), Jiayu 293 (common *Indica* rice with about 100 days, erect and compact), Jiazao 312 (common *Indica* rice with about 100d, erect and compact) and Jiazao324 (common *Indica* rice with about 105 days, erect and compact) were selected for investigation. They were seeded on June 2 and transplanted on June 25. The plants were provided with sufficient water during the whole growing period. Jiayu 293, Jiazao 312 and Jiazao 324 were harvested on Sept. 12, Xiushui 110 and Xieyou 9308 harvested on Oct. 15.

Testing site: A field experiment was conducted during June through October of 2002 at the Experiment Farm, Zhejiang University, Hangzhou (30°14′N, 120°10′E), China. A completely randomized design consisting of 60 plots of area 4.76×4.68 m was used. The single plant was transplanted with spacing by 0.13 m times 0.17 m.

Fertilizer treatments: The 60 plots represented three nitrogen levels (0, 120 and 240 kg N ha^{-1}, symbol N_0, N_1 and N_2, respectively) and four replications. The nitrogen fertilizer consists of 50% base fertilizer, 35% tiller fertilizer and 15% heading fertilizer to come into being no nitrogen fertilizer, proper one and superabundant one. In addition, 533.3 kg Ca $(H_2PO_4)_2$ ha^{-1} is base fertilizer, 300 kg KCl ha^{-1} heading fertilizer. The sandy loam paddy soil has the following properties: total N with 1.15 g kg^{-1}, quick-acting N with 188.5 mg kg^{-1}, total P with 1.21 g kg^{-1}, total K with 72.7 mg kg^{-1}, organic matter with 9.96 g kg^{-1} and with pH 6.78.

Measurement methods

Surveying instrument: The spectral radiometer was ASD (Analytical Spectral Device) FieldSpec Pro FR™ with range 350 to 2500 nm. The spectral sampling interval and resolution are 1.4 and 3 nm in the range 350-1000 nm and 2 and 10 nm in the range 1000-2500 nm, respectively. The spectrophotometer was BECKMAN DU-600 with 1nm spectral sampling interval and one-ten thousands OD value. The analytic software of data was SPSS10.0.

Spectral measurement

Canopy spectrum: The canopy spectral reflectance was determined at 10:00 am to 2:00 pm on a sunny day without cloud and wind at tiller stage, jointing stage, booting stage, heading stage, milking stage and maturing stage, respectively. The detector, about 0.70 m height from the canopy, is vertical down to opposite to a fixed position. A sampling spectrum consists of ten reading and ten sampling spectra averaged to represent the canopy mean spectral reflectance. Dark signal is subtracted and data compared to a standard white reference as spectral measurement.

The plants corresponding to the canopy spectra are sampled for determination of biophysical and biochemical parameters such as leaf, stem and panicle fresh mass, leaf area by leaf area meter, the contents of chlorophyll and carotenoid of leaves and panicles after the canopy spectral reflectance. Then they are killed green for 0.5 h at 105°C and dried at 70°C for determination of their dry mass.

Spectra of leaves and panicles: The leaves and panicles are sampled for spectral measurement and for their concentrations of chlorophyll and carotenoid at tiller stage, jointing stage, booting stage and 4 to 7 day interval after heading. The reflectance of uppermost unfolding leaves on the main stem of 3 group of rice plants with moderate growth selected from each plot are measured in laboratory by LC 1800 at a time. The panicle reflectance is measured in laboratory by ASD FieldSpec Pro FR™. The detector fixed by a triangle-rack, about 0.10 m height above the canopy, is vertical down to opposite the center of the sample on a black rubber with about zero reflectance. The angle of VOF was 8°. The light source, 50 W halogenate lamp, is 0.45 m and 70° from the sample surface. The spectral radiometer, controlling computer and light source are linked to a UPS. Dark signal is subtracted and data compared to a standard white reference before spectral measurement. A sampling spectrum consists of ten reading and ten sampling spectra averaged to represent the sample mean spectral reflectance.

Analysis of the concentrations of chlorophyll, carotenoid and Nitrogen: Leaf and panicle chlorophyll contents are analyzed with Arnon method, Leaf and panicle carotenoid contents analyzed with China Standard GB12291-90 method and nitrogen contents determined with Kjeldahl method after the plant materials are over-dried at 70°C for 36 h, ground and digested with H_2SO_4-H_2O_2.

RESULTS

Spectra of canopy, leaf and panicle: From Fig. 1a, the canopy reflectance increases in the red and blue region and decreases in the infrared region because that the leaf nutrient transfer to panicles and the lower leaves declined and fall off leading to change of leaf color and its internal structure, the canopy chlorophyll content in continuous decrease and LAI decrease after heading. As shown in Fig. 1b and c, the reflectance of the flag leaf were gradually getting bigger in the green region and bigger before milking and smaller after milking in the near infrared region and panicle reflectance are gradually getting bigger in the visible region and smaller in the near infrared region as the growth stages went on. But it had a strange increase. This seems to be related to its structure and change of biochemical components.

Correlative analysis of nitrogen content of rice panicle and paddy relating to the chlorophyll and nitrogen contents of leaves, stems and panicles: From Fig. 2a, the chlorophyll contents of flag leaves are gradually getting bigger from booting stage to heading stage (Aug. 22 ~ Sept. 4) and gradually getting smaller from the beginning of heading stage to the ending of heading stage (Sept. 4 ~ Sept. 8), then bigger from the ending of heading stage to milking stage (Sept. 8 ~ Sept. 20) and finally, smaller up to harvest. From Fig. 2b, the nitrogen contents of flag leaves are gradually getting smaller from the beginning of heading stage, but that of panicles are inverse.

Correlative analysis of nitrogen contents of rice panicle relating to the spectra of flag leaves and panicles: The correlation coefficients between the nitrogen contents (%) of panicles and hyperspectral reflectance of flag leaf and panicle, shown in Fig. 3, are calculated.

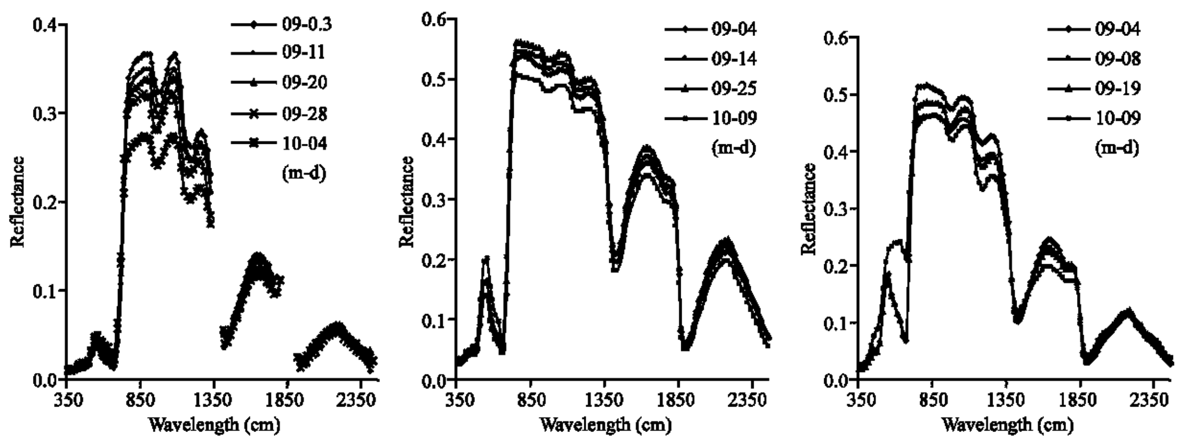

Fig. 1: Spectra of Xiushui 110 canopy, flag leaf and panicle at different development stages after heading (N1). (a): Canopy, (b): Flag leaf and (c): Panicle

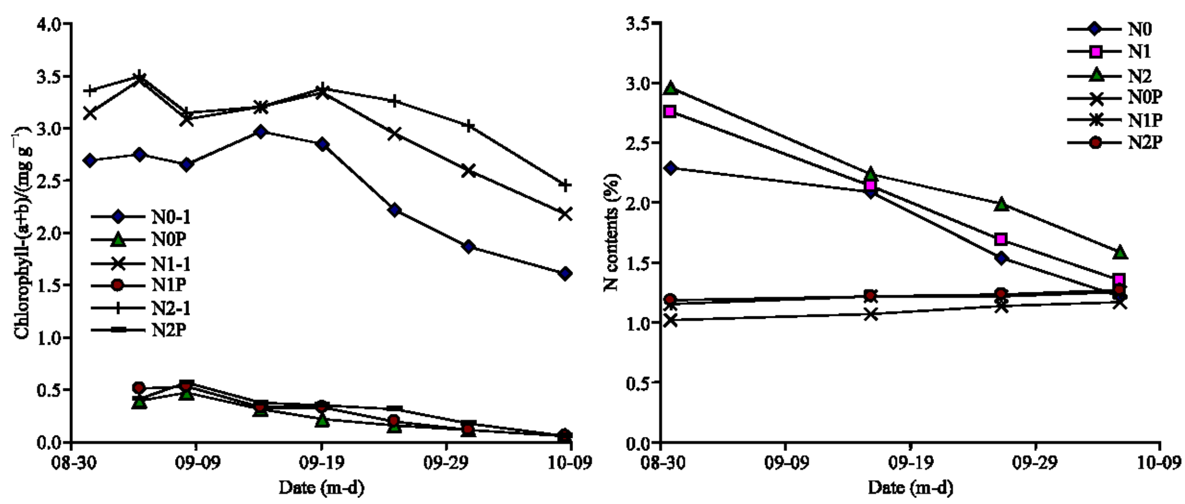

Fig. 2: Change of the contents of chlorophyll and nitrogen of flag leaves and panicles for Xiushui 110

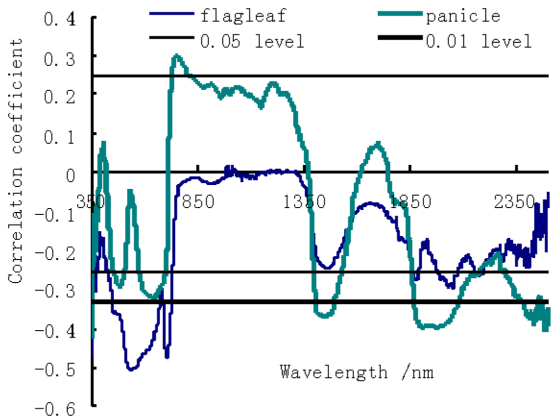

Fig. 3: Correlation coefficients between the nitrogen contents of panicles and spectral reflectance of flag leaf and panicle

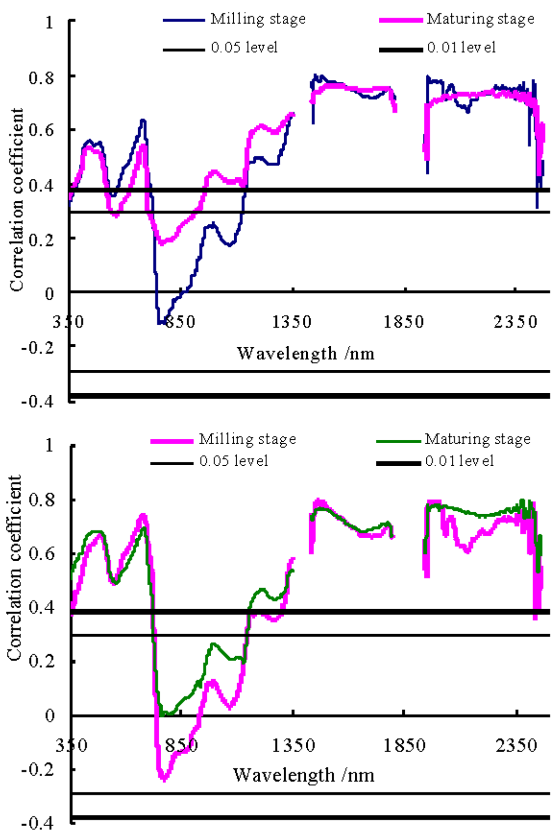

Fig. 4: Correlation coefficients between the nitrogen contents of panicles and paddy and canopy spectral reflectance (a) Panicle and (b) Paddy

There are significant correlation between the nitrogen contents of panicles and the spectra of flag leaves and panicles themselves in some wavebands. There are also significant correlation between the nitrogen contents of panicles and the first derivative spectra of flag leaves and panicles themselves in some wavebands. The maximum correlation coefficients R between the panicle nitrogen contents and the spectral reflectance ρ and their first derivative spectra Dλ of flag leaf and panicles are -0.508** at 534 nm, -0.397** at 1957 nm and 0.605** at 567 nm, 0.571** at 1654 nm, respectively.

Correlative analysis of nitrogen contents of rice panicle and paddy relating to the canopy spectra: The correlation coefficients between the nitrogen contents (%) of panicles, paddy and canopy spectral reflectance, shown in Fig. 4, are calculated at milking stage and maturing stage.

From Fig. 4a, there are preferably correlation between the nitrogen contents of panicles and their corresponding canopies spectra, where the correlation coefficients attained to positive significant levels in blue range, yellow range and short-wave infrared range. The maximum correlation coefficients R between the panicle nitrogen contents and the canopy spectral reflectance ρ, log (1/ρ), ratio vegetation index RVI and their first derivative spectra are 0.801** at 1452 nm, -0.794** at 2083 nm, -0.866** at ρ_{725}/ρ_{1483} and 0.830** at 827 nm at milking stage, 0.760** at 1529 nm, -0.763** at 1777 nm, -0.854** at ρ_{725}/ρ_{1529} and 0.798** at 675 nm at milking stage, respectively.

From Fig. 4b, preferred correlations can be found between the nitrogen contents of paddy and the canopies spectra at milking stage and maturing stage, where the correlation coefficients attained to positive significant levels in visible range and short-wave infrared range. But the correlation is bad at booting stage. The maximum correlation coefficients R between the panicle nitrogen contents and the canopy spectral reflectance ρ, log (1/ρ), ratio vegetation index RVI and their first derivative spectra are 0.798** at 1457 nm, -0.808** at 2076 nm, -0.866** at ρ_{739}/ρ_{1483} and 0.853** at 827 nm at milking stage, 0.801** at 2413 nm, -0.807** at 2449 nm, -0.847** at ρ_{736}/ρ_{1526} and 0.758** at 675 nm at milking stage, respectively.

The stepwise multiple regression equation between the nitrogen contents of panicles and paddy and the canopy spectral reflectance ρ_λ, the first derivative spectra D_λ and ratio vegetation index RVI are shown in Table 2.

From Table 2, it is known that over 80% of change of the nitrogen (or crude protein) contents of panicle and paddy are analyzed by stepwise multiple regression of ρ_λ, D_λ and RVI of canopy spectra at milking stage and over 75% of change of that at maturing stage. The estimating effect at milking stage is better than that at maturing stage. This is because the formation of nutrition component mainly depends on the milking process for rice seed.

Table 1: Correlation coefficients between nitrogen contents of paddy, panicles and that of leaves, stems (n = 45)

Chlorophyll and nitrogen contents of flag leaves, stems and panicles	Nitrogen contents of panicles (%)		Nitrogen contents of paddy (%)	
	Milking stage	Maturing stage	Milking stage	Maturing stage
Nitrogen contents of leaves (%)	0.597**	0.773**	0.439**	0.626**
Nitrogen contents of stems (%)	0.845**	0.840**	0.765**	0.777**
Nitrogen contents of flag leaves (%)	0.472**	0.390**		
Chlorophyll contents of flag leaves (mg g^{-1})	0.569**	0.498**		
% Stand for the percentage of mass				

**: Stand for significant difference at p<0.01 levels. The same as below

Table 2: The stepwise multiple regression equation between grain nitrogen contents and canopies spectra

Growth stage	Organ	Regression model	R^2	F
Milking stage	Panicle	$N_C = 0.987+25.362\rho_{1452}-6.605\rho_{705}-15.578\rho_{2352}+2.804\rho_{1949}$	0.834	50.2
		$=1.353+668.987\,D_{827}+1720.692\,D_{848}-359.310\,D_{988}$	0.836	56.4
		$=2.299-1.739\rho_{725}/\rho_{1483}+1.465\,\rho_{728}/\rho_{1505}$	0.806	82.1
	Paddy	$N_C = 1.011+37.829\rho_{1457}-33.353\rho_{2340}-39.085\rho_{400}+4.062\rho_{1946}$	0.878	72.3
		$=1.107+1023.662\,D_{827}+2136.953\,D_{427}+2466.884\,D_{673}$	0.884	104.2
		$=2.264-1.211\rho_{739}/\rho_{1483}+0.104\,\rho_{755}/\rho_{2118}+0.898\,\rho_{742}/\rho_{1505}$	0.823	59.6
Maturing stage	Panicle	$N_C =1.082+22.557\rho_{1529}-14.505\rho_{1794}-21.968\rho_{488}+9.868\rho_{2440}$	0.777	29.7
		$=0.872+1023.662\,D_{675}+523.019\,D_{1521}+219.291\,D_{1478}$	0.808	57.5
		$=2.620-1.275\rho_{725}/\rho_{1529}+0.424\,\rho_{720}/\rho_{1505}$	0.757	62.1
	Paddy	$N_C = 0.781+31.631\rho_{2413}-9.270\rho_{2450}-10.382\rho_{1802}+6.652\rho_{1778}$	0.783	36.1
		$=1.197+1965.369\,D_{675}+756.396\,D_{910}+300.105\,D_{965}$	0.790	42.4
		$=2.646-1.105\rho_{736}/\rho_{1526}+0.353\,\rho_{731}/\rho_{2105}$	0.768	76.1

N_C, ρ_λ, D_λ and $\rho_{\lambda1}/\rho_{\lambda2}$ Stand for grain nitrogen contents, canopy spectral reflectance, the first derivative spectrum and ratio vegetation index RVI, respectively. R^2, F Stand for the square of correlation coefficients r and statistical value F, respectively

DISCUSSION

From above analysis, there are significant correlation between the nitrogen contents of grain and the spectral reflectance ρ_λ and the first derivative spectra D_λ of flag leaves, panicles and canopy in some wavebands. There are also significant correlation between the nitrogen contents of grain and some RVI of the canopy spectra. The content of nitrogen or crude protein can be estimated by the spectral characteristics of these sensitive wavebands in theoretically. It is known that over 75% of change of the nitrogen (or crude protein) contents of panicle and paddy are analyzed by stepwise multiple regression of ρ_λ, D_λ and RVI of canopy spectra. The estimating effect by derivative spectra is better than that by original spectra. This is because the absorbing apices of different chemical components, such as protein, starch and cellulose in rice grain, overlap one another in infrared range (Curran, 1989). The derivative spectra, however, is in favor of separating these absorbing characteristics by excavating their change trend.

In addition, the estimation of nitrogen contents of rice grain from canopy spectra at milking stage is better than that at maturing stage. This is because the formation of nutrition component mainly depends on the milking process for rice grain. The nutrition component of rice grain mostly comes from transformation of nutrition saved in stems and leaves before heading. The most crude protein has already been transferred to grain from stems and leaves after maturing. That the contents of nitrogen and chlorophyll in leaf evidently reduce the results in the relatively weak contribution for leaf chlorophyll is positively correlated to the nitrogen content by spectral reflectance of canopy.

Of course, the nitrogen contents of rice grains are related not only to fertilization and growth but also to variety, development season and planting methods (Wopereis-Pura *et al*., 2002; Cheng *et al*., 2003). It is impossible to accurately estimate the nitrogen contents of rice grains only from its canopy spectra. This study would provide a basic idea and practical method to monitor rice quality by remote sensing.

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