ISSN 1009 – 3095 *Journal of Zhejiang University*(*SCIENCE*) V.2,No.3,P.289 – 293,July – Sep.,2001
http://www.chinainfo.gov.cn/periodical; http://www.zju.edu.cn;
http://lib.zju.edu.cn/English; http://www.zjupress.com

VERTICAL LEAF SPECTRAL VARIATION AS AN INDICATOR OF NITROGEN NUTRITION STATUS IN RICE PLANTS*

ZHOU Qi-fa(周启发)[1], WANG Ren-chao(王人潮)[2], SHEN Zhang-quan(沈掌泉)[2],
WANG Xiu-zhen(王秀珍)[2], YU Jun-ping(于军平)[1]

([1] *College of Life Science, Zhejiang University, Hangzhou 310012, China*)
([2] *College of Resource and Environment, Zhejiang University, Hangzhou 310029, China*)

Received Oct.29, 2000; revision accepted Jan.8, 2001

Abstract: A field experiment was conducted to study the response of individual leaf spectral reflectance to five levels of nitrogen fertilizer treatments in rice(*Oriza sativa L.*) plants. Sampling was combined through a rice canopy at upper, medium and low levels for biomass, nitrogen and water content measurements with spectral signals from the leaves. The vertical gradients of leaf biomass, nitrogen and water contents were associated with the nitrogen availability during tillering, panicle formation, initial heading and heading. Rice plants treated with the lowest rate of N could be characterized with the lowest value of gradient in leaf biomass and leaf water content and the highest value of gradient in leaf N concentration. A spectral gradient of single reflectance(R), ratio(RVI) and normalized difference(ND) of two individual reflectances was defined as this yielded a better relationship between the spectral data and leaf nitrogen concentration. The results suggested the spectral gradients may be used as an improved diagnostic tool for nitrogen status.

Key words: nitrogen status, reflectance, rice, spectral gradient
Document code: A **CLC number:** Q945.1

INTRODUCTION

Visible and near infrared reflection spectroscopy was proved in recent years to offer the possibility of obtaining a rapid, non-destructive estimation of plant nitrogen contents(Thomas et al., 1972; Hinzman et al., 1986; Takebe et al., 1990; Filella et al., 1995; Blackmer et al., 1994, 1996; Ladha et al., 1998; Ruano-Ramos et al., 1999). In these studies, the reflectance measured from canopies, individual leaves or other plant parts and its spectral indices were correlated with plant nitrogen contents. This correlation may vary with plant species, plant part, growth stage and a range of environmental factors as plant spectral reflection is affected by numerous factors (Filella et al., 1995; Carter, 1993) and this reduces greatly the practical application value of the technique. For practical use, spectral index response, more specifically to nitrogen nutrition status, must be established.

In rice plants, nitrogen remobilizes from old leaves into younger leaves when nitrogen is deficient(Liu, 1982), so it could be deduced that nitrogen deficiency causes vertical N concentration gradient within a plant. The relation between N concentration gradient and the available N resource has drawn relatively little attention of researchers in comparison with the extensive studies of N partitioning inside canopies and within a plant in relation to leaf irradiance(Drouet et al., 1999). Since leaf spectral reflectance is correlated with leaf N contents, a spectral gradient that could exist within a rice plant deficient in nitrogen may give more specific implications of nitrogen nutrition status in comparison with spectral reflectance measured from plant canopy or single plant part, which changes in response to numerous stress agents.

In this paper, we report results of our inves-

* Project (49771056) supported by Natural Science Foundation of China(NSFC).

tigation on the vertical spectral variation's relation to nitrogen nutrition status in rice plants.

MATERIALS AND METHODS

A field experiment was conducted from July through October of 1999 at the Experimental Farm, Zhejiang University, Hangzhou (30°10′N, 120°12′E). Rice (*Oriza sativa L.*) cv. 'Xiushui 9363' was selected for the investigation. The sandy loam paddy soil was Inceptsols with pH 5.66, organic matter 16.6 $g \cdot kg^{-1}$ and total N 1.02 $g \cdot kg^{-1}$. A completely randomized design consisting of twenty 4m × 5m plots was used. The 20 plots represented five nitrogen levels (0, 45, 105, 165 and N 225 $kg \cdot ha^{-1}$) and four replications. The plants were transplanted on 25 July 1999 with 13.3 cm × 16.7 cm spacing.

The second uppermost leaf, second lowest leaf and the leaf midway between them were sampled for determination of biomass, water content and nitrogen content and for spectral measurement on 19 August (tillering), 28 August (panicle formation), 12 September (initial heading) and 25 September 1999 (heading). Leaf reflectance was measured by a Field Spec model spectroscopy (a product of Irricrop Technologies Pty. Ltd.). The GVI window was 333 to 1056 nm on the X axis and 0.00 to 1.10 nm on the Y axis; and the wavelength step was 1.4 nm. VNIR dark signal was subtracted and data compared to a white $BaSO_4$ reference. Eight replicate spectral measurements per nitrogen level treatment were made. After spectral measurement, the leaves were immediately determined for biomass, water content and N concentration. Leaf water content was determined by weighing the biomass before and after oven drying at 70℃ for 24h. Leaf N content was determined with the Kjeldahl method after the plant materials were oven dried at 70℃ for 24h, ground and digested with $H_2SO_4 - H_2O_2$.

All crop and reflectance data were analyzed using conventional statistical analysis. The spectral indices were calculated as 1) R550 = (R546 + R548 + R549 + R550)/4; 2) R680 = (R678 + R679 + R681)/3; 3) R928 = (R925 + R927 + R928)/3; 4) RVI1 = R550/R680; 5) RVI2 = R928/R680; 6) ND1 = (R550 − R680)/(R550 + R680) and 7) ND2 = (R928 − R680)/(R928 + R680) for analysis of relation between spectral characteristics and nitrogen nutrition in rice plants (Zhou and Wang, 1993), where R indicated reflectance at certain wavelength, RVI indicated ratio and ND indicated normalized difference of two individual reflectances.

RESULTS AND DISCUSSIONS

As shown in Table 1, leaf biomass and nitrogen content increased with N rate increasing at various stages. The biomass of the second uppermost leaves, compared to that of the medium position leaves and second lowest leaves, was more responsive to N rates. Higher N rates caused higher water contents in the second uppermost leaves while responses of water contents in the medium position and the second lowest leaves to N rates were more complex.

The results (Table 1) show clearly leaf biomass and N content decreased with the leaf position becoming low at each N rate treatment and at various stages. The leaf water content showed similar changing at tillering, but it increased as the leaf position became lower at all the later growth stages. To quantify the vertical leaf gradients of biomass, N content and water content, we express the gradient as $G_p = (P_u - P_l) + (P_m - P_l) = P_u + P_m - 2P_l$, where G_p denotes the gradient of parameter P as biomass, nitrogen content and water content, u, m and l denote respectively the second uppermost leaf, medium position leaf and the second lowest leaf. The calculated gradient values are presented in Table 2. As the growth stage progressed, the gradient of leaf nitrogen content increased, that of leaf water content decreased and that of leaf biomass increased to maximum at initial heading and started to decreased at heading. While as the N rate increased, the gradients of leaf N content and water content decreased and that of biomass increased. This result may be of general significance in crop N nutrition status diagnosis as it is found that gradients of leaf biomass, N content and water content were associated closely with the nitrogen nutrition status and the plant growth stages, although light environment and developmental difference could be the major influencing factors of the gradients (Grindlay, 1977). Plants with the lowest level of N could be characterized by the lowest gradient of leaf biomass, highest gradient of leaf nitrogen and lowest gradient of water content. Conventionally, the leaf critical concentration is used for nutrition diagnosis, but it should be regarded as a range of values rather than a precise figure (Smith, 1986). If this is so, it greatly reduces the value of plant analysis (Scaife, 1988). The leaf gradients could characterize the plant nitrogen status as they may be more specifically responsive to the available N resource in comparison to N concentrations in plant.

Table 1 Leaf biomass, nitrogen concentration and water content at three leaf positions of rice plants treated with different N rate at various growth stages*

Date	N rate (kg•ha^{-1})	Biomass (g/leaf)			Leaf N content (%)			Leaf water content (%)		
		U	M	L**	U	M	L	U	M	L
19 Aug 1999	0	0.17a	0.11a	0.08a	3.50a	3.00a	2.88a	72.79ab	71.12ab	68.89a***
	45	0.17a	0.13b	0.10b	3.50a	3.40b	3.21b	72.47a	70.54a	68.46a
	105	0.23b	0.15c	0.10b	3.60a	3.48b	3.23b	73.87b	71.52b	69.80b
	165	0.24bc	0.12b	0.10b	4.14b	3.72c	3.61c	73.99b	71.65b	69.50b
	225	0.25c	0.15c	0.10b	4.11b	3.86c	3.76c	73.87b	70.45a	70.27b
28 Aug 1999	0	0.30a	0.14a	0.10a	2.83a	2.40a	2.09a	71.68a	71.63a	72.64a
	45	0.32a	0.15a	0.14b	3.46bc	3.00b	2.71b	72.42ab	72.94b	73.01ab
	105	0.36b	0.17b	0.14b	3.40b	3.32c	2.96c	72.54b	73.65bc	74.42b
	165	0.40c	0.21c	0.16c	3.51c	3.53d	3.38d	74.46c	74.32c	74.92a
	225	0.38c	0.17c	0.14b	3.44b	3.65e	3.42d	74.06c	73.27b	73.68b
12 Sept 1999	0	0.24a	0.25a	0.12a	2.70a	2.07a	1.36a	67.70a	73.59b	76.27d
	45	0.32b	0.25a	0.12a	2.91b	2.63b	1.99b	68.84ab	72.94a	75.42c
	105	0.37c	0.26ab	0.14b	3.31c	3.18c	2.45c	69.48b	74.46b	75.61c
	165	0.37c	0.27b	0.16c	3.30c	3.27c	2.78d	69.20b	72.11a	73.25b
	225	0.39d	0.24a	0.12a	3.55d	3.42d	3.36e	70.64c	74.25b	74.65a
25 Sept 1999	0	0.26a	0.30c	0.18ab	2.42a	1.78a	0.86a	64.64a	72.60b	73.22bc
	45	0.31b	0.24a	0.17ab	2.85b	2.09b	1.47b	66.55b	68.95a	73.91c
	105	0.36c	0.28b	0.16a	2.97c	2.50c	2.08c	66.48b	71.47b	73.07bc
	165	0.33b	0.28b	0.15a	3.45e	2.94d	2.65d	67.69c	69.35a	72.82b
	225	0.39c	0.29b	0.19b	3.19d	2.96d	2.58d	67.15bc	69.23a	71.81a

* Values are averages of six replications for leaf biomass and water content, three replications for leaf nitrogen content.

** U denotes the second uppermost leaf, M denotes the medium position leaf and L denotes the second lowest leaf.

*** Measurements followed by different letters are significantly different ($P = 0.05$) by Duncan's multiple range test.

Table 2 The vertical gradients of leaf biomass, N content and water content in rice plants treated with different N rate at various growth stages

N rate (kg/ha)	Gradient on 19 Aug			Gradient on 28 Aug			Gradient on 12 Sept			Gradient on 25 Sept		
	Biomass (g/leaf)	N (%)	Water (%)	Biomass (g/leaf)	N (%)	Water (%)	Biomass (g/leaf)	N (%)	Water (%)	Biomass (g/leaf)	N (%)	Water (%)
0	0.12	0.74	6.13	0.24	1.05	−1.97	0.25	2.05	−11.25	0.20	2.48	−9.20
45	0.10	0.48	6.09	0.19	1.04	−0.66	0.33	1.56	−9.06	0.21	2.00	−12.32
105	0.18	0.62	5.79	0.25	0.80	−2.65	0.35	1.59	−7.28	0.32	1.31	−8.19
165	0.16	0.64	6.64	0.29	0.28	−1.06	0.32	1.01	−5.19	0.31	1.09	−8.60
225	0.20	0.45	3.78	0.27	0.25	−0.03	0.39	0.25	−4.41	0.30	0.99	−7.24

Table 3 The distribution of reflectance maximum/minima number of 120 leaf spectr a at maxium/minima wavelengths at various times

Date of measurement	Maximum/minima wavelength(nm)									
	546	548	549	550	678	679	681	925	927	928
19 Aug 1999	23	36	25	36	38	33	49	42	35	43
28 Aug 1999	7	13	33	67	19	41	60	40	34	46
12 Sept 1999	5	23	30	62	9	24	87	44	35	41
25 Sept 1999	1	7	30	82	2	8	110	36	50	34

All of the plant leaves measured had a similar reflectance pattern: maximum in the vicinity of 550 nm and 928 nm and minima in the vicinity of 680 nm (The raw spectral data are not shown as the data size is too large for publication). We found the maximum and minima shift (see Table 3), even among the leaves from the same plant position and same N rate treatment, which had not been clearly demonstrated before. In the visible regions (around 550 nm and 680 nm), the reflectance maximum/minima shifted toward longer wavelengths as the growth stage progressed while in the near infrared region, the shift of maximum was not affected significantly by the growth stages. A possible explanation for the shift toward longer wavelengths as the growth stage progressed is the effect of senescence (Guyot, 1990).

Single reflectance values at the individual wavelengths (550, 680 and 928 nm) were more highly correlated to the leaf nitrogen content than to their spectral indices (Table 4), which disagreed with the previous results (Zhou et al., 1993; Filella et al., 1995; Wang et al., 1998). Maybe our use of a combination of leaves from different positions in analyzing the correlation between the spectral index and nitrogen content decrease the coefficients between the spectral index and nitrogen content in this study. Wavelength regions that appeared to be the best indicators were centered around 550 nm and 680 nm, confirming the result of Filella et al. (1995) and that of Blackmer et al. (1996).

Spectral variation with leaf position was observed in each plant measured in this study. We define the vertical leaf spectral gradient as $G_{sp} = SP_u + SP_m - 2SP_l$ in the same way we define the vertical leaf N gradient defined, where G_{sp} denotes spectral gradient of a spectral index as R550, RVI1 and ND1, u denotes the second uppermost leaf, m denotes the medium position leaf and l the second lowest leaf. The higher coefficients between the vertical leaf spectral gradient and the vertical leaf gradient of N content (Table 5) comparing with those between the spectral indices and the nitrogen contents suggested the spectral variation could be used as an indicator of plant nitrogen nutrition status. The gradients of ND1 + ND2 were most highly correlated with the gradient of nitrogen contents, which may be attributed to the specific response of this spectral index to the chlorophyll content with sensitive wavelength being 550 nm and 680 nm (Al-Abbas, 1974) and leaf water status with sensitive wavelength being 900 – 950 nm (Riedell et al., 1999) associated closely with the nitrogen nutrition status. The subtraction among spectral index of leaf at different position from the same plant in the spectral gradient analysis may reduce greatly the effects of some biological and environmental factors on the leaf spectra, so the spectral gradients could be highly related to gradients of leaf biological features (e.g. N concentration). Further studies must be conducted for confirm of the results in different plant species, cultivars and in different plant growth conditions.

Table 4 Coefficients of determination between leaf spectral indices and nitrogen contents at various growth stages of rice plants treated with different N rate ($n = 45$)

Dates	r							
	R550	R680	R928	RVI1	RVI2	ND1	ND2	ND1 + ND2
19 Aug 1999	0.49*	−0.52*	−0.39*	0.18	−0.12	0.067	−0.11	0.16
28 Aug 1999	0.59*	−0.41*	0.54*	0.18	−0.17	0.55*	0.57*	0.63*
12 Sept 1999	0.47*	−0.65*	0.18	0.17	0.69*	0.42*	0.71*	0.58*
25 Sept 1999	0.51*	−0.58*	0.40*	−0.11	0.49*	−0.31	0.47*	−0.11

* Denotes significant ($P = 0.05$)

Table 5 Coefficients of determination between vertical leaf gradients of spectral indices and vertical gradients of nitrogen contents at various growth stages of rice plants treated with different N rate ($n = 15$)

Dates	r							
	GR550	GR680	GR928	GRVI1	GRVI2	GND1	GND2	GND1 + ND2
19 Aug 1999	0.84*	0.90*	−0.18	0.89*	−0.54*	−0.89*	−0.56*	−0.96*
28 Aug 1999	0.41	0.45	0.25	0.75*	−0.59*	−0.91*	−0.93*	−0.91*
12 Sept 1999	0.77*	0.72*	0.72*	0.60*	−0.77*	−0.80*	−0.83*	−0.82*
25 Sept 1999	0.69*	0.78*	−0.19	0.69*	−0.82*	−0.82*	−0.59*	−0.81*

* Denotes significant($P = 0.05$)

CONCLUSIONS

Gradients of leaf parameters (biomass, water content, N concentration and spectral index) were proved to exist and be associated closely with nitrogen availability in rice plants. When interpreting plant nitrogen status in rice by using leaf spectral data, spectral gradient within a plant may be more indicative than spectral index from individual leaves.

References

Al-Abbas A.H., Barr R., Hall J.D., et al., 1974. Spectra of normal and nutrient-deficient maize leaves. *Agron. J.* **66**: 16 – 20.

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

Blackmer, T.M, Schepers, J.S., Varvel, G.E., et al., 1996. Nitrogen deficiency detection using reflected shortwave radiation from irrigated corn canopies. *Agron. J.* **88**: 1 – 5.

Carter, G.A., 1993. Responses of leaf spectral reflectance to plant stress. *Am. J. Bot.* **80**: 239 – 243.

Drouet, J-L., and Bonhomme, R., 1999. Do variations in local leaf irradiance explain changes to leaf nitrogen within row maize canopies? *Annals of Botany* **84**: 61 – 69.

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

Guyot, G., 1990. Optical properties of vegetative canopies. p.19 – 44. *In*: Application of remote sensing in agriculture. M D Steven and J A Clark Eds. Butterworths Publ., London, p.19 – 44.

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

Ladha, J.K., Tirol-Padre, A., Punzalan, G.C., et al., 1998. Nondestructive estimation of shoot nitrogen in different rice genotypes. *Agron. J.* **90**: 33 – 40.

Liu, Z.Y., 1982. A Collection of Illustrative Plates for Nutritional Disorder in Principal Crops in China. Agricultural Publishing Press, Beijing (in Chinese).

Riedell, W.E., Blackmer, T.M., 1999. Leaf reflectance spectra of cereal aphid-damaged wheat. *Crop Sci.* **39**: 1835 – 1840.

Ruano-Ramos, A., García-Ciadad, A., García – Criado, B., 1999. Determination of nitrogen and ash contents in total herbage and botanical components of grassland systems with near infra-red spectroscopy. *J. Sci. Food Agric.* **79**: 137 – 143.

Scaife, A., 1988 Derivation of critical nutrient concentrations for growth rate from data from field experiments. *Plant and Soil.* **109**: 159 – 169.

Smith, F.W., 1986 Interpretation of plant analysis: concepts and principles. *In*: Plant Analysis: an Interpretation Manual. D J Reuter and J B Robinson Eds. Inkata Press, Melbourne.

Takebe, M., Yoneyama, T., Inada, K., et al., 1990. Spectral reflectance ratio of rice canopy for estimating crop nitrogen status. *Plant and Soil.* **122**.295 – 297.

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

Walburg, G., Bauer, M.E., Daughtry, C.S.T., et al., 1982. Effects of nitrogen nutrition on the growth, yield, and reflectance characteristics of corn canopies. *Agron. J.* **74**: 677 – 683.

Wang, R.C., Wang, K., Shen, Z.Q., 1998. Feasibility of field evaluation of rice nitrogen status from reflectance spectra of canopy. *Pedosphere* **8**: 121 – 126.

Zhou, Q.F., and Wang, R.C., 1993. The relation between leaf spectral characteristics and nitrogen level in early rice. *J. Zhejiang Agric. Univ.* **19**(Sup): 15 – 22.