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Detection of nitrogen-overfertilized rice plants with leaf positional difference in hyperspectral vegetation index*

Qi-fa ZHOU[†1], Zhan-yu LIU[2], Jing-feng HUANG[†‡2]

([1]*College of Life Sciences, Zhejiang University, Hangzhou 310058, China*)

([2]*College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310029, China*)

[†]E-mail: zqifa2002@yahoo.com; hjf@zju.edu.cn

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Abstract: The main objective of this work was to compare the applicability of the single leaf (the uppermost leaf L1 and the third uppermost leaf L3) modified simple ratio (mSR_{705} index) and the leaf positional difference in the vegetation index between L1 and L3 (mSR_{705L1}–mSR_{705L3}) in detecting nitrogen (N)-overfertilized rice plants. A field experiment consisting of three rice genotypes and five N fertilization levels (0, 75, 180, 285, and 390 kg N/ha) was conducted at Xiaoshan, Hangzhou, Zhejiang Province, China in 2008. The hyperspectral reflectance (350–2500 nm) and the chlorophyll concentration (ChlC) of L1 and L3 were measured at different stages. The mSR_{705L1} and mSR_{705L3} indices appeared not to be highly sensitive to the N rates, especially when the N rate was high (above 180 kg N/ha). The mean mSR_{705L1}–mSR_{705L3} across the genotypes increased significantly (P<0.05) or considerably from 180 to 285 kg N/ha treatment and from 285 to 390 kg N/ha treatment at all the stages. Also, use of the difference (mSR_{705L1}–mSR_{705L3}) greatly reduced the influence of the stages and genotypes in assessing the N status with reflectance data. The results of this study show that the N-overfertilized rice plants can be effectively detected with the leaf positional difference in the mSR_{705} index.

Key words: Rice, Nitrogen (N), Overfertilization, Leaf position, Hyperspectral reflectance
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1 Introduction

Rice is the main food staple for more than 50% of the world's population (Fageria and Baligar, 2003). Nitrogen (N) fertilizer is one of the most important inputs for rice production in the world. Increasing the N fertilizer rate for rice plants, however, does not always increase grain yield, due to diminishing returns. The excessive use of N fertilizers poses potential adverse environmental and health concerns (Bohlool *et al*., 1992), and increases the incidence of foliar pathogens and plant lodging (Stroppiana *et al*., 2009). Farmers tend to use more N fertilizer than needed mainly because of its subsidized price and its immediate visible impact on plant growth and leaf color (Islam *et al*., 2007). Approximately 30% of the N used as fertilizer in the world is consumed in China, rice crop use accounting for about 37% use (Peng *et al*., 2002). Therefore, rapid and real time detection of N overfertilization during rice production could be very helpful for site-specific N management.

In recent years, spectral measurements have been used for rapid and non-destructive estimation of crop N status. The use of radiometric data for N estimation has been reported over a wide range of crops (Blackmer *et al*., 1996; Boegh *et al*., 2002; Hansen and Schjoerring, 2003). Controversy exists, however, in the use of radiometric data for rice (Shibayama and Akiyama, 1986; Takebe *et al*., 1990; Xue *et al*., 2004; Nguyen and Lee, 2006; Zhu *et al*., 2007; Yi *et al*., 2007; Stroppiana *et al*., 2009). Estimating plant N

‡ Corresponding author

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status by spectral data is sometimes difficult as values may vary according to plant age, location, cultivar selection, and soil characteristic (Spaner *et al*., 2005). Particularly, N estimation for crops of high N and chlorophyll (Chl) contents using reflectance data may be difficult because crop reflectance may be insensitive to crops of high N and chlorophyll contents due to the saturated light adsorption in the chlorophyll-adsorption region (Thomas and Gausman, 1977).

N availability profoundly influences the distribution of leaf N, and thus leaf chlorophyll within a canopy profile as N is a highly variable element in the plant. Wang *et al*. (2006) showed that the leaf positional differences in N concentration in response to varied N rates could be a useful indication of plant N status in rice. It was implied that the leaf positional differences in N-sensitive or chlorophyll-sensitive hyperspectral indices might be useful for reducing the influences of some factors (e.g., locations and cultivar) on the critical hyperspectral index, thereby allowing for detection of plants with high N and chlorophyll contents by spectral data.

Previous research has paid little attention to detecting N overfertilization when assessing plant N status with the spectral approach. The main objectives of this work are (1) to test the applicability of the selected chlorophyll-sensitive hyperspectral index in detecting N-overfertilized rice plants and (2) to explore the feasibility of using the leaf positional differences in the chlorophyll-sensitive hyperspectral index for detecting N-overfertilized rice plants.

2 Materials and methods

2.1 Field experiment

A field experiment was conducted on a sandy loam soil from June to October in 2008 at the research farm of Xiaoshan Agricultural Sciences Research Institute, Xiaoshan, Hangzhou, Zhejiang Province, China (30°20′ N, 120°31′ E). Prior to planting, the original soil had 13.1 g/kg organic C, 5.6 mg/kg bicarbonate extractable P, 35.2 mg/kg exchangeable K, and 1.26 g/kg total N with pH 7.5 (soil:water 1:1 (w/v)). Three genotypes, Yongyou 8, Zhongzheyou 1, and Zhejing 22 of rice (*Oryza sativa* L.), were selected for study. A random block design was used with the three genotypes and five N rates (0, 75, 180, 285, and 390 kg N/ha). Forty percent of the N fertilizer (urea) was applied at pre-planting, 30% at the tillering stage, and 30% at initial heading. Each treatment was replicated three times. The plants were transplanted on July 8, 2008 and harvested on October 20, 2008.

2.2 Spectral measurement

One whole rice plant from each plot was collected at panicle initiation, heading and milk stages, and transported to the lab for leaf reflectance measurements. The reflectance of the single leaf (the uppermost leaf L1 and the third uppermost leaf L3) on a randomly selected main stem was measured with an integrating sphere (Model LI-1800, LiCor Inc., Lincoln, NE, USA) coupled to a FieldSpec® model spectral radiometer (Analytical Spectral Devices, Boulder, CO, USA) in the wavelength range of 350–2500 nm around the midpoint of each leaf, each measurement of a leaf being the average of ten scans. The white reference was taken before each spectral measurement.

2.3 Leaf chlorophyll concentration determination

After spectral measurements, leaves of 0.20 g from the middle part of each leaf were sampled for determination of leaf chlorophyll concentration (ChlC). The Chl a and Chl b concentrations per unit mass were measured spectrophotometrically using 80% (v/v) acetone as a solvent, employing the equations of Lichtenhaler and Wellburn (1983).

2.4 Data analysis

Modified simple ratio [$mSR_{705}=(R_{750}-R_{445})/(R_{705}-R_{445})$, where R_{750}, R_{445}, and R_{705} are the reflectance at 750, 445, and 705 nm, respectively (Sims and Gamon, 2002)] was used as the vegetation index (VI) for retrieving leaf ChlC. This VI was selected because it can reduce the effect of differences in leaf surface reflectance and is relatively insensitive to leaf structure (Sims and Gamon, 2002).

Statistical analysis was done with SPSS 16.0 (Chicago, IL, USA). An analysis of variance (ANOVA) was done to determine the significance of the effects of genotypes, stages, N level, and leaf position on the spectral index and ChlC. Factorial analyses of variance were conducted to determine the significance of the effects of genotypes, stages, and N levels on the VI and ChlC differences between L1 and

L3. A linear contrast test was used to test the significance of the differences among the five N levels.

For the convenience of illustration, 0, 75, 180, 285, and 390 kg N/ha are designated as N0, N1, N2, N3, and N4, respectively, VIs of L1 and L3 are expressed as VI_{L1} and VI_{L3}, respectively, and the leaf ChlCs in L1 and L3 are expressed as $ChlC_{L1}$ and $ChlC_{L3}$, respectively. The leaf positional difference in the VI between L1 and L3 is expressed as $mSR_{705L1}-mSR_{705L3}$ or $VI_{L1}-VI_{L3}$, and the ChlC difference between L1 and L3 is expressed as $ChlC_{L1}-ChlC_{L3}$.

3 Results

3.1 Response of leaf chlorophyll to N rate

The leaf ChlC ranged from 0.20 to 6.18 mg/g, indicating the leaf ChlC range was wide due to the genotypes, N treatments, stages, and leaf positions. As shown in Table 1, the genotype, the stage, and the N rate significantly affected (P<0.05) the leaf ChlC. A significant (P<0.05) variation due to stage×genotype, stage×N rate, N rate×leaf position, N rate×genotype, stage×genotype×N rate, and genotype×N rate×leaf position interactions was also observed, although the effect of the leaf position on the leaf ChlC was insignificant (P>0.05). The N rate also affected significantly (P<0.05) $ChlC_{L1}-ChlC_{L3}$ (Table 2). As presented in Fig. 1 and Table 3, both the mean $ChlC_{L1}$ and the mean $ChlC_{L3}$ across the three stages and the three genotypes and across the three genotypes at each stage tended to increase with the N rate, although the N2 treatment appeared to have the highest $ChlC_{L1}$ among the N treatments at the milk stage. In particular,

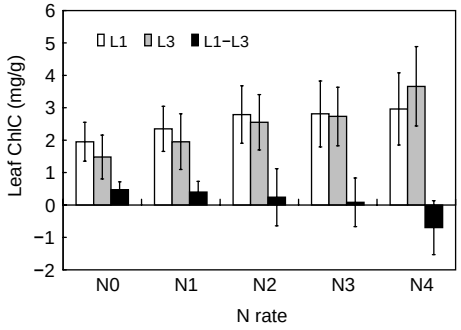

Fig. 1 Mean leaf chlorophyll concentrations (ChlCs) in response to the five N rates for the three genotypes at Xiaoshan, Hangzhou, Zhejiang Province, China in 2008
Values are means of all three genotypes and three stages for a given N level (n=27). Bars indicate standard errors

Table 1 Analysis of variance of the effects of stage (S), genotype (G), N fertilization rate (N), and leaf position (LP) on mSR_{705} index and leaf chlorophyll concentration (ChlC) of three rice genotypes grown under five N rates at Xiaoshan, Hangzhou, Zhejiang Province, China in 2008

Source	*df*	mSR_{705}	ChlC
S	2	20.16***	127.60***
G	2	72.25***	41.25***
N	4	27.61***	67.28***
LP	1	3.18	1.50
S×G	4	9.07***	7.15***
S×N	8	2.29*	4.23***
S×LP	2	0.69	1.75
G×N	8	2.15*	3.98***
G×LP	2	0.52	0.02
N×LP	4	7.59***	10.86***
S×G×N	16	1.77*	2.21*
S×G×LP	4	1.20	0.41
S×N×LP	8	0.73	0.51
G×N×LP	8	0.56	2.34*
S×G×N×LP	16	0.30	0.51
Error	180		

*, **, and ***: significant at 0.05, 0.01, and 0.001 levels, respectively

Table 2 Analysis of variance of the effects of stage (S), genotype (G), and N fertilization rate (N) on the leaf positional difference values in mSR_{705} and leaf chlorophyll concentration (ChlC) between the uppermost leaf (L1) and the third uppermost leaf (L3) of three rice genotypes grown under five N rates at Xiaoshan, Hangzhou, Zhejiang Province, China in 2008

Source	*df*	$mSR_{705L1}-mSR_{705L3}$	$ChlC_{L1}-ChlC_{L3}$
S	2	2.16	4.25*
G	2	1.48	0.16
N	4	21.98***	20.14***
S×G	4	3.73**	0.95
S×N	8	2.55*	5.34***
G×N	8	1.65	3.90**
S×G×N	16	0.87	0.87
Error	90		

*, **, and ***: significant at 0.05, 0.01, and 0.001 levels, respectively

the N4 treatment had the highest $ChlC_{L3}$ and the lowest $ChlC_{L1}-ChlC_{L3}$ among the five N levels at all the stages (Table 3).

3.2 Response of the VI to the N rate

As shown in Table 1, the genotype, stage, and N rate affected significantly (P<0.05) the mSR_{705} index. A significant (P<0.05) variation due to stage×genotype,

Table 3 Mean mSR_{705} and leaf chlorophyll concentration (ChlC) values of the uppermost leaf (L1) and the third uppermost leaf (L3) and the difference values between L1 and L3 in response to the five N rates for the three genotypes at different stages at Xiaoshan, Hangzhou, Zhejiang Province, China in 2008

Stage	N level	mSR_{705L1}	mSR_{705L3}	$mSR_{705L1}-mSR_{705L3}$	$ChlC_{L1}$ (mg/g)	$ChlC_{L3}$ (mg/g)	$ChlC_{L1}-ChlC_{L3}$ (mg/g)
Panicle initiation	N0	2.39a	2.27a	0.12a	2.27a	1.85a	0.42a
	N1	2.65b	2.51b	0.14a	2.76a	2.56b	0.20a
	N2	2.62b	2.64b	−0.02b	2.68a	3.03c	−0.35b
	N3	2.59b	2.67b	−0.08b	2.66a	3.02c	−0.36b
	N4	2.59b	2.72b	−0.13b	2.96b	3.33d	−0.37b
Heading	N0	2.69a	2.36a	0.33a	2.27a	1.89a	0.38a
	N1	2.62a	2.36a	0.26a	2.62a	2.30a	0.32a
	N2	3.06b	2.96b	0.10b	3.23b	3.07b	0.16a
	N3	2.93b	2.91b	0.02b	3.67cd	3.10b	0.57a
	N4	2.89b	3.06b	−0.15c	3.97d	4.63c	−0.66b
Milk	N0	2.32a	2.04a	0.28a	1.31a	0.72a	0.59a
	N1	2.57a	2.18a	0.39a	1.67ab	0.98a	0.69a
	N2	2.83b	2.67b	0.16a	2.48c	1.53b	0.95a
	N3	2.71b	2.72b	−0.01b	2.08bc	2.07bc	0.01b
	N4	2.46a	2.96b	−0.50c	1.94b	3.02c	−1.08c

Values are means of all three genotypes for a given N level (n=9). Comparison of the means was done by linear contrast test. Values denoted with different letters are significant among the five N levels at the 0.05 level

stage×N rate, N rate×leaf position, N rate×genotype, and stage×genotype×N rate interactions was also observed for the VI, although the effect of the leaf position on the VI was insignificant (P>0.05). The results indicated that the VI varied greatly with the four factors.

The N rate had a significant (P<0.05) effect on $mSR_{705L1}-mSR_{705L3}$, but the effects of stage and genotype on $mSR_{705L1}-mSR_{705L3}$ became insignificant (P>0.05) (Table 2). The results indicated that the influences of stage and genotype could be greatly reduced when $mSR_{705L1}-mSR_{705L3}$ instead of mSR_{705L1} or mSR_{705L3} was used in assessing the N status by reflectance data.

As shown in Table 3, the values of mSR_{705} tended to increased with the N level from N0 to N2 at all the stages for both L1 and L3. The differences in the VI were considerable, although not always significant, between N0 and N1 treatments. The mean values of the VI were not significantly (P>0.05) different among N2, N3, and N4 except that the N4 treatment had significantly (P<0.05) lower IV than N2 or N3 treatment at milk stage, indicating that the VI was not sensitive to the high N levels (above N2).

The mean $mSR_{705L1}-mSR_{705L3}$ across the genotypes decreased from N2 treatment to N4 treatment at all the stages, and was significantly (P<0.05) higher at heading and milk in N2 and N3 treatments than in N4 treatment. The difference in the mean $mSR_{705L1}-mSR_{705L3}$ was considerable, although not always significant, between N2 and N3 treatments at all the stages. The $mSR_{705L1}-mSR_{705L3}$ appeared, however, to be poor in separating N0 and N1 treatments. The results indicated that $mSR_{705L1}-mSR_{705L3}$ was sensitive to the high N levels, although it was not sensitive to changes at low N levels (N0 and N1). The N-overfertilized rice plants (N4 treatment) were characterized with the lowest and most negative values of $mSR_{705L1}-mSR_{705L3}$.

The ChlC-mSR_{705} relationships were positive and significant (P<0.05), but not robust as the coefficient was 0.49 and 0.64 (n=135), respectively, in L1 and L3, implying that the single leaf VI was not a robust estimator of the leaf ChlC across the genotypes and stages. Fig. 2 shows that the mSR_{705} index tends to increase with the leaf ChlC, but becomes insensitive to changes in high leaf ChlC (above 3.5 mg/g).

4 Discussion

The chlorophylls contain a large proportion of total leaf N. Therefore measurements of ChlC can provide an accurate indirect assessment of plant

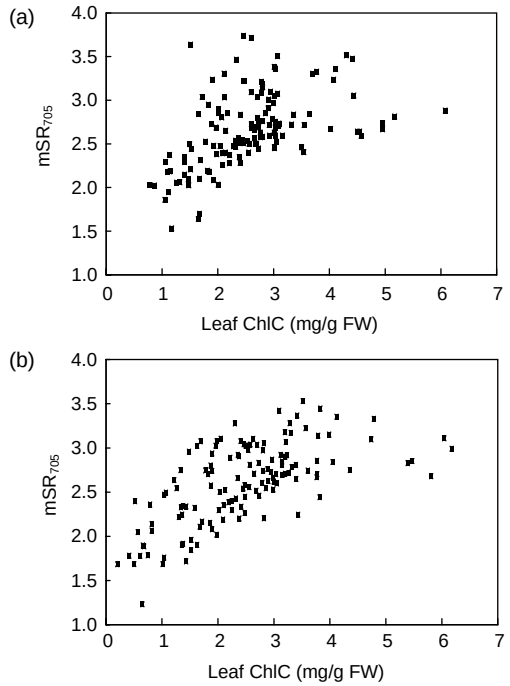

Fig. 2 Relationship between mSR_{705} and leaf chlorophyll concentration (ChlC)
(a) The first uppermost leaf L1; (b) The third uppermost leaf L3

nutrient status (Moran *et al*., 2000). The results of this study further confirm that leaf ChlC can be used to assess rice N status. At the milk stage, N2 treatment appeared to have the highest ChlC in the flag leaves, but N3 and N4 treatments still had a remarkably higher ChlC than N2 treatment in L3, which might be attributed to a feedback mechanism in response to N remobilization from the leaves to the grain.

The single leaf hyperspectral index appeared not to be highly sensitive to the N rates, especially under high N conditions. This might be partly explained by the fact that VI might not be sensitive to high ChlC, since a relatively low content of chlorophyll is sufficient to saturate absorption in the chlorophyll-adsorption region (Thomas and Gausman, 1977).

The leaf position did not statistically influence mSR_{705} or leaf ChlC. The N rate×leaf position interaction, however, significantly (P<0.05) influenced both the mSR_{705} and leaf ChlC (Table 1), and the N rate was found to significantly (P<0.05) influence both mSR_{705L1}−mSR_{705L3} and $ChlC_{L1}$−$ChlC_{L3}$ (Table 2). Therefore, the VI_{L1}−VI_{L3} can be used to assess rice N status. The VI_{L1}−VI_{L3} appeared to be useful, while both the VI_{L1} and the VI_{L3} performed poorly in separating N2, N3, and N4 treatments, possibly because the use of the VI_{L1}−VI_{L3}, instead of the single leaf hyperspectral index, can greatly reduce the influence of the genotypes and the stages, and relatively enlarge the VI differences among the high N treatments (Table 3). The VI_{L1}−VI_{L3} appeared, however, not to be as effective as the single leaf VI in separating N0 and N1 treatments, corresponding to the insignificant (P>0.05) difference in the $ChlC_{L1}$−$ChlC_{L3}$ between the two treatments. The results suggest that combined analyses of the VI_{L1}, VI_{L3}, and VI_{L1}−VI_{L3} are needed when assessing plant N status with hyperspectral reflectance data.

The ChlC-VI relationship was not robust in both L1 and L3. The relationship would be improved if the ChlC was expressed on the basis of area. The wide range of leaf ChlC might also influence the ChlC-VI relationship, since the VI was not sensitive to high leaf ChlC.

5 Conclusions

The single leaf hyperspectral index mSR_{705} appeared not to be highly sensitive to the N rates, especially when the N rate was high (above N2). The leaf positional differences in the VI appear to be sensitive to the high N rates at all the stages, which could be explained by the fact that the influence of stages and genotypes can be greatly reduced, and that the treatment differences among the high N level treatments are relatively enlarged by using the VI_{L1}−VI_{L3} instead of the VI_{L1} or the VI_{L3} in assessing the N status via reflectance data. The N-overfertilized rice plant (N4 treatment) was characterized with the lowest and most negative value of the VI_{L1}−VI_{L3} at all the stages. The results in this study suggest that the leaf positional differences in the VI can be used to effectively detect the rice plants with N overfertilization.

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