Accurate identification of nitrogen fertilizer application of paddy rice using laser-induced fluorescence combined with support vector machine

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ABSTRACT

To identify accurately the doses of nitrogen (N) fertilizer and improve the photosynthetic efficiency of paddy rice, laser induced fluorescence (LIF) technique combined with the support vector machine (SVM) and principal component analysis (PCA) is proposed in this paper. The LIF technology, in which the ultraviolet light (355 nm) is applied as an excitation light source, is employed to measure fluorescence spectra of paddy rice. These fluorescence spectra demonstrate that the fluorescence spectral characteristics of paddy rice leaves with different doses of N fertilizer have distinct differences from each other. Then, PCA and SVM are implemented to extract the features of fluorescence spectra and to recognize different doses of N fertilizer, respectively. The overall recognition accuracy can reach 95%. The results show that the LIF technology combined with PCA and SVM is a convenient, rapid, and sensitive diagnostic method for detecting N levels of paddy rice. Thus, it will also be convenient for farmers to manage accurately their fertilization strategies.

Keywords: remote sensing; macronutrient; multivariate analysis; chlorophyll content; *Oryza sativa*

Paddy rice is a major crop in China. Therefore, it is important to boost paddy rice yields and obtain high-quality grains. At present, most investigators mainly focus on researching crop tolerance and optimal growth conditions to improve the yields of paddy rice. In addition, farmers also tend to use nitrogen (N) fertilizer to boost the production of paddy rice. So far, it has been found that there are 16 necessary nutrient elements that influence the yield of crops, which are carbon, hydrogen, oxygen, nitrogen, phosphorus and potassium, etc. However, N level of crops is one of the most important nutrient diagnosis indicators that governs canopy carbon assimilation, which is tightly connected with chlorophyll concentration of leaves and influences importantly the photosynthetic capacity of crops (Green et al. 2003, Stroppiana et al. 2009). Thus, in order to improve the production of paddy rice, farmers tend to apply excessively N fertilizer, which will result in disease-resistant drop, pyricularia oryzae, sheath blight, etc. All of these causes can impact seriously on the yields and quality of paddy rice (Li et al. 2007). Thus, technologies and methods, which are employed to detect N status of crops, are indispensable.

In the last decades, many techniques and methods have been developed to detect the N status of crops, such as passive remote sensing (Gitelson et al. 1999), active reflectance measurement (Daughtry et al. 2000) and passive chlorophyll fluorescence

(Blackburn 1998), etc. Passive remote sensing technology is based on the fact that the different nutrient stress of vegetation has different spectral reflectance at characteristic wavelengths. Thus, it is a significant tool to analyse the nutrient stress of crops (Gong et al. 2012). However, the instrument is expensive and insensitive for variation of nutrient stress of crops at early stages of growth. Thus, there have been numerous attempts to find more efficient methods for monitoring the nutrient stress of crops. The passive fluorescence was employed successfully in the nutrient stress of crops in the field of remote sensing. The principle of passive fluorescence is that leaf pigments can ray part or all of its absorbed energy as fluorescence at longer wavelengths after exposed to photons of a certain wavelength (McMurtrey et al. 1994). Nevertheless, this technology is restricted by many factors such as the signal to noise ratio (SNR), experimental conditions, etc.

As a mature technique, LIF has the capability to monitor the nutrient stress of vegetation. Thus, LIF technology has been applied widely in physiological stress of plant and other fields (Sherson et al. 2010, Stelzer 2015). For example, it was successfully employed to obtain the growth status, monitor nutrient stress in sugar beet, corn and wheat such as the deficit of major plant nutrients N, P and K (McMurtrey et al. 1994), and to assess environmental influence on soybean, such as drought (Živčák et al. 2008), high temperature (Brestič et al. 2012), and light stress. Moreover, Yang et al. (2015) has shown that different N content will also influence the fluorescence spectral characteristics of paddy rice. Thus, fluorescence spectroscopy has the potential to become a useful method for detection of different doses of N fertilizer in the field. In addition, it has the superiority of rapid response and less impact on the environment, and the most important is that it is a non-invasive technology (Živčák et al. 2014). However, these studies just focus on the spectral characteristics and few investigations have been done on influence of different doses of N fertilizer on fluorescence spectral characteristics of paddy rice.

In this paper, the system of LIF with a pulsed Nd:YAG laser emitting at a wavelength of 355 nm was utilized as an excitation source. The fluorescence spectral data with different doses of N fertilizer, which were measured using this system, are conserved in the spectral database. The principal component analysis (PCA) and support vector machine (SVM) were used to analyse qualitatively the N levels of paddy rice. It is found that the identification rate of N levels can reach 95%. Thus, nutrient stress resulting from the absence of N fertilizer can be monitored by using the LIF technology combined with PCA and SVM. Thereby, they could be applied to offer advice for farmers on their N fertilizer strategies and guide them to immediately rectify the imbalance of nutrient stress in that area of the field. Besides, it is possible to effectively decrease environmental pollution caused by over-fertilization and avoid low-yielding caused by inadequate fertilization.

MATERIAL AND METHODS

Study areas and site description. The experimental fields of paddy rice are situated in Junchuan County, Suizhou city, in the province of Hubei, China. The district is located in the middle reaches of the Yangze River which is well-known as the Jianghan Plain (Song et al. 2011). The paddy rice was planted by using mouldboard plough tillage and the cultivar was Yongyou 4949, which was seeded on 27 April, 2014. There were 69 experimental fields and six different levels of N fertilization corresponding to 270 kg/ha, which is found appropriate to satisfy the N requirements of paddy rice and is recommended by the local farm extension service, no nitrogen, insufficient level (15% less and 30% less), and excessive level (15% more and 30% more), respectively, as shown in the Table 1.

Table 1. Paddy rice field nitrogen (N) treatments (SQ – standard N rate)

Label	N0	N1	N2	N3	N4	N5
N fertilization rate	–	SQ – 30%	SQ – 15%	SQ	SQ + 15%	SQ + 30%
N applied (kg/ha)	–	189	229.5	270	310.5	351

N0 – no nitrogen; N1 – 189; N2 – 229.5; N3 – 270; N4 – 310.5; N5 – 351 kg N/ha

In addition to controlling fertilizer rate, plastic films were also used to enclose the ridges of the experimental field to eliminate the influence of water seepage. The urea N fertilizer was proportionally distributed in four splits: 30% at pre-planting, 20% at tillering, 25% at shooting and 25% at booting. The samples of paddy rice leaf, i.e. the 2nd leaves from the top and all fully expanded, were disruptively sampled by randomly cutting six leaves with three replicates at different positions of each experimental field. In order to eliminate the effect of photosynthesis on the N level of paddy rice leaf, all samples were stored in a refrigerator at −20°C before measurement. A subsample was randomly chosen from the samples to measure the fluorescence spectra of paddy rice leaf (Li et al. 2014). In addition, the chlorophyll content of paddy rice was obtained spectrophotometrically with acetone extract, and computed employing the equation of Lichtenthaler (1987). The standard Kjeldahl method was employed to measure the N content (Živčák et al. 2014).

Principles. The fluorescence intensity is one of the most important indices for detection of plant nutrient stress and can be presented as Günther et al. (1994):

$$F(\lambda) = I_0(\lambda_L) \times C \times \alpha(\lambda_L) \times \phi \times \int e^{-(k + k')z} dz \qquad (1)$$

Where: $I_0(\lambda_L)$ – intensity of excitation light; $\alpha(\lambda_L)$ – specific absorption coefficient of chlorophyll at incident light wavelength; ϕ – fluorescence efficiency at detection wavelength; k, k' – extinction coefficients at incident light and detection wavelength; C – concentration of pigment in the leaf. The exponential function is integrated over the leaf thickness d.

Laser induced fluorescence (LIF) instrument. Figure 1 shows the scheme of the LIF instrument, which is composed of three main parts: the laser source part, optical receiver system, and the signal acquisition assembly. The excitation source is a laser of Nd:YAG, which emits pulses at 355 nm, and was operated at 20 Hz with the output power and the width per pulse being 1.5 mJ and 5 ns, respectively. A telescope of Maksutov-Cassegrain is used to collect fluorescence signal of paddy rice.

The fluorescence induced was transmitted into the fiber optics and then entered the spectrometer, and a 355 nm cut-off filter was placed in front of the fiber optics to stop reflected light from the laser entering the fiber optics. The fluorescence signal as a function of wavelength was acquired by using ICCD, and PC is utilized to store and process fluorescence data. In this paper, the scans range of spectrometer is 360–800 nm with the sampling interval of 0.5 nm. Before measurement, the samples of paddy rice leaves were pasted on black cards to avoid the influence of sundries. To ensure the same location of the measurement of every sample, the measuring position is fixed in 3 cm from top of paddy rice leaf. Fluorescence data are processed by using Matlab (a mathematical tool).

Methods. All experimental data consisted of 216 measurements from all samples (six different levels of N fertilizer, and two growth stages of paddy rice, tillering and shooting). Firstly, the PCA was utilized to extract the feature vectors and to reduce the dimensions of the fluorescence spectra data sets. Then, all feature vectors were randomly separated into two parts as follows: 72% of the feature vectors as the training set to train the SVM model and the other part containing 28% as the validation set to test the accuracy of the model. Before analysis, spectral radiant correction was utilized to calibrate the fluorescence intensity, and then wavelet transform and moving window polynomial fitting were employed to denoise and smooth it, respectively.

RESULTS AND DISCUSSION

As shown in Table 2, different doses of N fertilizers result in differences in the N levels in paddy rice. In addition, the N content increases with
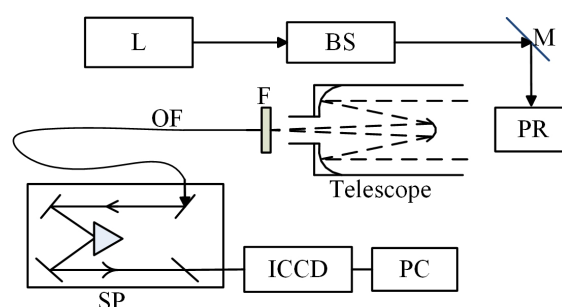

Figure 1. Schema of the laser induced fluorescence system. L – 355 nm laser; BS – 10 times beam expander at 355 nm; M – completely reflecting mirror; PR – paddy rice sample; F – 355 nm cut-off filter; OF – optics fiber; SP – spectrograph; ICCD – intensified charge coupled device; PC – personal computer

Table 2. Nitrogen (N) content of paddy rice at different growth stages

Label	Tillering stage		Shooting stage	
	chlorophyll (mg/g)	N content (%)	chlorophyll (mg/g)	N content (%)
N0	1.96	1.63	1.75	1.04
N1	2.59	2.12	2.47	1.49
N2	2.84	2.14	2.58	1.57
N3	3.11	2.25	2.72	1.78
N4	3.19	2.41	2.86	1.99
N5	3.22	2.49	3.1	2.09

N0 – no nitrogen; N1 – 189; N2 – 229.5; N3 – 270; N4 – 310.5; N5 – 351 kg N/ha

the increase of N fertilizer application (Živčák et al. 2014).

Owing to the fluctuations in fluorescence that occur from 440–523 nm and are probably either a build-up or shortage of compounds (such as NADPH and vitamin K), it will result in different fluorescence reactions. Fluorescence spectra of paddy rice was normalized to 1 at 460 nm (Figure 2). Figure 2 shows that chlorophyll fluorescence spectra range from 360–800 nm and centering wavelength at 685 nm and 740 nm (Gitelson et al. 1999). The two peaks are contributed by antennae chlorophyll of Photosystem I (740 nm) and Photosystem II (685 nm), respectively. As concluded by Satio et al. (1998), chlorophyll which included the chlorophyll *a* and *b* is tightly dependent on the N levels of paddy rice (Živčák et al. 2014, Yang et al. 2015).

From the above discussions, it is effortless to find that the fluorescence spectra of paddy rice leaf with different doses of N fertilizer at different growth stage can be easily separated from each other (Figure 2). To identify different doses of N fertilizers of paddy rice, PCA and SVM that were utilized to extract the feature vectors and to analyse the fluorescence spectral data, respectively. Firstly, PCA is used to decrease the dimensionality of spectral data. The result demonstrated that 88.44% of the total variance can be explained by using the first two factors of Z-scores (74.6% and 13.84%, respectively) (Figure 3).

Then, SVM was proposed to identify different N levels of paddy rice based on the fluorescence spectra. 72% of fluorescence data were used to train SVM model and the other 28% of fluorescence spectra were applied to validate the model. The results of testing are listed in Table 3.

From Table 3, it can be concluded that the total recognition accuracy could reach up to 95% for six different doses of N fertilizers by using the
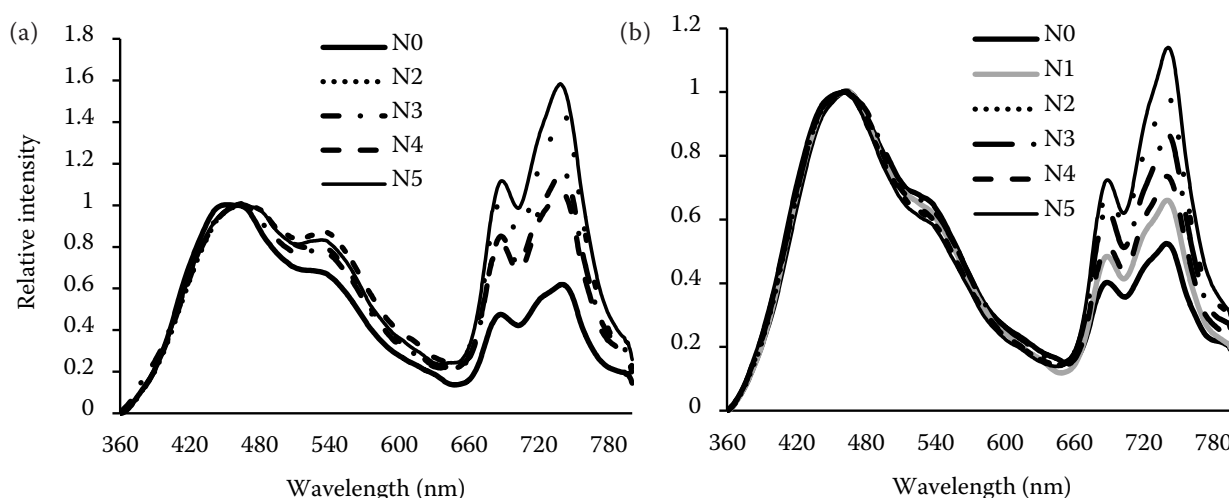

Figure 2. The normalized relative fluorescence intensity subjected to six different doses of nitrogen (N) fertilizer at different growth stages. (a) tillering stage; (b) shooting stage. N0 – no nitrogen; N1 – 189; N2 – 229.5; N3 – 270; N4 – 310.5; N5 – 351 kg N/ha
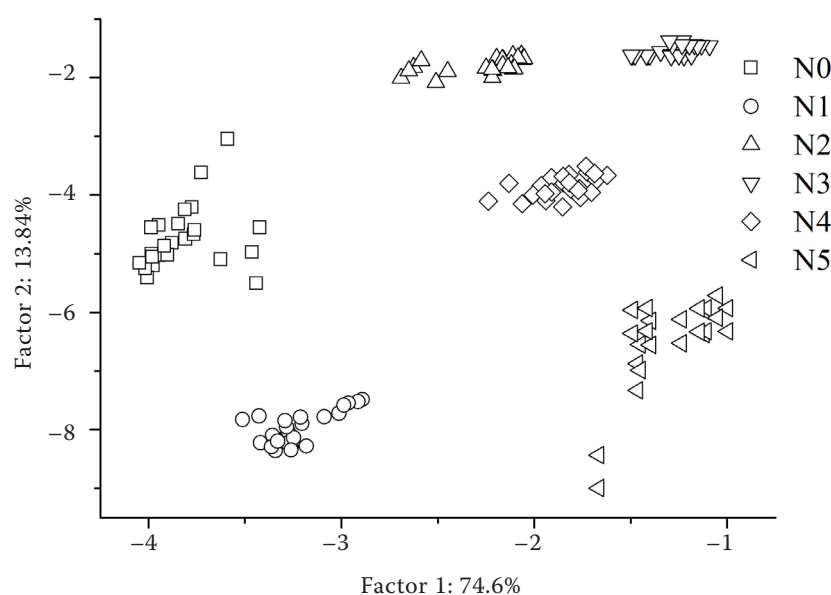
Figure 3. Principal component analysis of six different levels of nitrogen (N) fertilizer. N0 – no nitrogen; N1 – 189; N2 – 229.5; N3 – 270; N4 – 310.5; N5 – 351 kg N/ha

SVM combined with PCA based on the fluorescence spectral characteristics. This paper thus can provide the guidance for the decision-making of farmers in their N fertilization strategies and guide them to rapidly rectify the imbalance of N content in the area of the field. Thus, the LIF technology combined with multivariate analysis will be helpful in monitoring of the N status of vegetation.

In conclusion, the numerical results have shown that the N content of paddy rice increases with the increasing dose of N fertilizer. Then, the LIF technology under the ultraviolet excitation wavelength 355 nm combined with PCA and SVM may be utilized to identify the N fertilizer levels of paddy rice. The experimental results demonstrated that the fluorescence spectra have tight positive correlation with the dose of N fertilizer.

The approach has the advantage of being rapid, sensitive and non-invasive, and was shown to be successful in accurate identification of the dose of paddy rice N fertilizer at different growth stages. The overall recognition accuracy can reach up to 95%. Thus, it could provide support for the decision-making of farmers in their N fertilizer strategies that can effectively boost paddy rice yields. In addition, the environmental pollution caused by over-fertilization may also be validly reduced. Moreover, if more fluorescence spectra and field measurements are obtained, the model can be then suggested for on-time monitoring levels of N fertilizer.

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Table 3. Recognition rate of different levels of nitrogen (N) fertilizer

Label	N0	N1	N2	N3	N4	N5
Number of test	10	10	10	10	10	10
Correct identification number	10	9	9	10	9	10
Error identification number	0	1	1	0	1	0
Identification rate (%)	100	90	90	100	90	100

N0 – no nitrogen; N1 – 189; N2 – 229.5; N3 – 270; N4 – 310.5; N5 – 351 kg N/ha

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