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Procedia Engineering 15 (2011) 4403 – 4408

Procedia Engineering

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Advanced in Control Engineering and Information Science

Monitoring Leaf Chlorophyll Fluorescence with Spectral Reflectance in Rice (*Oryza sativa* L.)

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Abstract

Non-destructive and rapid monitoring methods for leaf chlorophyll fluorescence (CF) of rice is significance in estimating rice growing, enhancing more efficient application of agrochemicals and reducing yield loss. The present study investigated the use of physiological indices calculated from spectral reflectance as potential indicators of rice (*Oryza sativa* L.) photosynthetic apparatus affected by root oxygen and rice types. The experiments showed that spectral reflectance and leaf chlorophyll fluorescence changed with different treatments. Principal component analysis (PCA) method was used to select the key spectral ranges of leaf chlorophyll fluorescence. Based on the PCA, 7 key spectral indices (SI) were selected to monitor leaf CF. In these SI, $(R_{680} - R_{935})/(R_{680} + R_{935})$ and R_{680}/R_{935} have higher R and lower RMSE, could be used for monitoring chlorophyll fluorescence, such as Fo, Fm, Fv/Fm, and Fv/Fo, while ΦPSII and NPQ could be detected by $(R_{800} - R_{445})/(R_{800} - R_{680})$ and $(R_{780} - R_{710})/(R_{780} - R_{680})$ respectively. Therefore, it was implied that chlorophyll fluorescence of rice response to root oxygen stress could be detected by remote sensing.

Keywords: Chlorophyll fluorescence; Leaf; Photosynthesis, Rice; Spectral reflectance

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# Contribution equal to first author

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doi:10.1016/j.proeng.2011.08.827

1. introduction

Chlorophyll fluorescence (CF) is red and far-red light that is produced in plant photosynthetic tissues upon excitation with natural or artificial light in the visible spectrum. Production of CF is one of the ways in which plant chloroplasts harmlessly dissipate light energy that is in excess of the needs of photosynthesis [1-3]. So, CF has been widely used to detect physiological status of plants under different abiotic stress, for example in the presence of nitrogen deficiency, cold, drought, etc [4, 5].

Previous studies have described reflectance indices potentially related to incremental effects of fluorescence emission as an addition to leaf and canopy reflected signal [6-10]. At the laboratory level, results in diurnal studies have shown that optical indices R_{680}/R_{630}, R_{685}/R_{630}, R_{687}/R_{630} and R_{690}/R_{630} were sensitive to Fv/Fm measured in leaves [7, 8]. At the canopy level in the laboratory and under natural light conditions, red edge indices were assessed for detection of chlorophyll fluorescence, including red edge spectral derivative indices D_{730}/D_{706}, DP_{21} ($D\lambda p/D_{703}$), where λp is the inflection point of the reflectance spectrum in the red edge spectral region [10]. At the airborne and far-field scales, solar-induced fluorescence signal on apparent reflectance could be obtained from airborne sensors Reflective Optics System Imaging Spectrometer (ROSIS) and Compact Airborne Spectrographic Imager (CASI) based on the in-filling of fluorescence in the 760 nm atmospheric oxygen absorption band [8]. These researches imply the potentially important applications in CF detection using passive spectral remote sensing methods.

Rice is one of important crops in the world and yield loss occurs every year for the nitrogen deficiency, disease, pest, etc. Monitoring of rice growing, which allows for a more efficient application of agrochemicals, could lead to reduce rice yield loss [11, 12]. However, to our knowledge, there has been few report yet related to the use of a ground-based radiometer for the measurement of rice CF. Therefore, objectives of this study were as follows: (1) to evaluate the effects of root oxygen stress on functionality of rice (*Oryza sativa* L.) photosynthetic apparatus, measured by CF and spectral reflectance; (2) to assess the ability of spectral reflectance to detect CF under root oxygen stress.

2. Material and methods

2.1. Experimental design

The experiment was conducted at the greenhouse of China National Rice Research Institute, Hangzhou, China, which is located at 30°05′N, 119°56′ E. Rice seeds were sown on May 2010. The experiment was a randomized complete block design with three replications. Three typical rice (*Oryza sativa* L.) cultivars 'Zhongzheyou 1', 'Zhonghan 221', and 'Shenshui' were used as the main plot treatments and five root oxygen concentrations, i.e., CK (O_0), 2.0 (O_1), 4.0(O_2), 6.0 (O_3) and 8.0 (O_4) mg/l, with an area of 2 m^2 (2 m × 1 m) for each plot.

2.2. Measurements of chlorophyll fluorescence

Leaf chlorophyll fluorescence measurements were conducted on the top leaf of each plant using a pulse-amplitude-modulation fluorometer (PAM 2100, H. Walz, Effeltrich, Germany). Before measuring chlorophyll (Chl) fluorescence parameters (FPs), leaves were put in dark-adapted state for 30 min using light exclusion clips. The following chlorophyll fluorescence yields (FYs) were measured: minimum Chl FY in the dark-adapted state (F_0), minimum Chl FY in the light-adapted state (F'_0), maximum Chl FY in the dark-adapted state (F_m), maximum Chl FY in the light-adapted state (F'_m), and steady-state Chl FY in the light-adapted state (F_s). Using these parameters, the following ratios were calculated: maximum PSII photochemical efficiency, $F_v/F_m = (F_m - F_0)/F_m$, effective quantum yield of photochemical energy conversion in PSII, ΦPSII = $(F'_m - F_s)/F'_m$, non-photochemical dissipation of absorbed energy, NPQ = $(F_m - F'_m)/F'_m$.

2.3. Measurements of spectral reflectance

Spectral measurements were taken using an AvaSpec-2048 spectrometer (Avantes inc., Netherlands). This spectrometer is fitted with a 25 field of view fiber optics, operating in the 200–1100 nm spectral region with a sampling interval of 1.4 nm and spectral resolution of 1.2 nm. The measurements were carried out from a height of 1.0 m above the canopy and 0.44 m view diameter under clear sky conditions between 10:00 h and 14:00 h (Beijing local time). Measurements of vegetation radiance were made at 10 sample sites in each plot, with each sample from averaging 20 scans at an optimized integration time. The saved spectrum file contained continuous spectral reflectance at 0.6 nm step over the band region of 200–1100 nm. A panel radiance measurement was taken before and after the vegetation measurement by two scans each time.

2.4. Data analysis

Spectral data were firstly exported from binary by using the manufacturer's program of AvaSoft 7.3 for USB 2. Reflectance data were firstly smoothed with a five-point moving average to suppress instrumental and environmental noise in the data before the data were further analyzed. By using principal component analysis (PCA) method, the key spectral index (SI) was selected. Then, correlation analyses between the chlorophyll fluorescence and SI were conducted in the Statistical Package for the Social Sciences (SPSS 12.0) and the precision of regression model was assessed by correlationship coefficient (R) and root mean square error (RMSE).

3. Results and Discussion

Table 1 indicated that leaf chlorophyll fluorescence (Fo, Fm, Fv/Fm, Fv/Fo, ΦPSII, and NPQ) of rice was significantly affected by root oxygen and rice type. As figure 1 showed, spectral reflectance in rice also changed with wave length. Principal component analysis (PCA) method was used to select the sensitive spectral ranges of leaf chlorophyll fluorescence, (Fig. 2). PCA results showed that 87% of spectral information and 13% of spectral information were explained by the first principal component and the second principal component respectively.

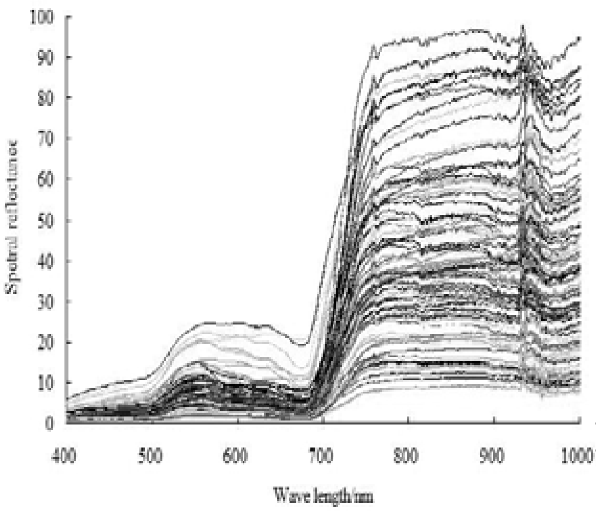
Based on PCA computing, 7 key spectral indices were selected (Tab. 2). Showing from Table 3, R_{800}/R_{680}, $(R_{800}-R_{680})/(R_{800}+R_{680})$, $(R_{800}-R_{445})/(R_{800}-R_{680})$, $(R_{680}-R_{935})/(R_{680}+R_{935})$, and R_{680}/R_{935} were significantly correlated (P<0.05) with Fo, Fm, Fv/Fm and Fv/Fo, while $(R_{780}-R_{710})/(R_{780}-R_{680})$ and $(R_{800}-R_{445})/(R_{800}-R_{680})$ significantly correlated with ΦPSII. Additionally, $(R_{780}-R_{710})/(R_{780}-R_{680})$ and $(R_{850}-R_{710}/(R_{850}-R_{680})$ were significantly correlated with NPQ. The best spectral indices, which should have higher correlationship coefficient (R) and lower root mean square error (RMSE), could be used for detecting chlorophyll fluorescence. Table 2 and Table 3 demonstrated that $(R_{680}-R_{935})/(R_{680}+R_{935})$ and R_{680}/R_{935} have higher R and lower RMSE, which could be used for monitoring chlorophyll fluorescence, such as Fo, Fm, Fv/Fm, and Fv/Fo, and ΦPSII and NPQ could be detected by $(R_{800}-R_{445})/(R_{800}-R_{680})$ and $(R_{780}-R_{710})/(R_{780}-R_{680})$ respectively.

In this study, photosynthetic apparatus of *Oryza sativa* L. was damaged to a certain extent for root oxygen stress, as observed from leaf chlorophyll fluorescence such as Fo, Fm, Fv/Fm, Fv/Fo, and PSII (Tab.1). In fact, upon exposure to O_3, plants show a decline in photosynthesis during the strain phase, before visible symptoms occur [19]. This decline is associated to a change in the repartition of absorbed energy in three competing processes: light reactions of photosynthesis and the dissipation pathways of excess energy (i.e. fluorescence and NPQ). In this study, leaf chlorophyll fluorescence was significantly correlated with R_{800}/R_{680}, $(R_{800}-R_{680})/(R_{800}+R_{680})$, $(R_{800}-R_{445})/(R_{800}-R_{680})$, $(R_{680}-R_{935})/(R_{680}+R_{935})$, and R_{680}/R_{935}, while $(R_{780}-R_{710})/(R_{780}-R_{680})$ and $(R_{850}-R_{710}/(R_{850}-R_{680})$ were significantly correlated with NPQ. It was implied that chlorophyll fluorescence of rice response to root oxygen stress could be detected by remote sensing.

Table 1. Statistical summary showing the results of two factorial ANOVAs for the effect of root oxygen, rice and root oxygen × rice type on the leaf chlorophyll fluorescence

Source	Fo		Fm		Fv/Fm		Fv/Fo		ΦPSII		NPQ	
	df	F	df	F	df	F	df	F	df	F	df	F
(A) Root oxygen	1,25	6.8**	1,25	7.3**	1,25	8.0**	1,25	9.3**	1,25	5.5**	1,25	8.2**
(B) Rice type	1,25	5.4**	1,25	3.6*	1,25	3.1*	1,25	5.1**	1,25	4.7**	1,25	5.9**
(C) Root oxygen × Rice type	1,25	5.9**	1,25	6.0**	1,25	5.2**	1,25	7.8**	1,25	6.3**	1,25	4.4**

*P<0.05 **P<0.01



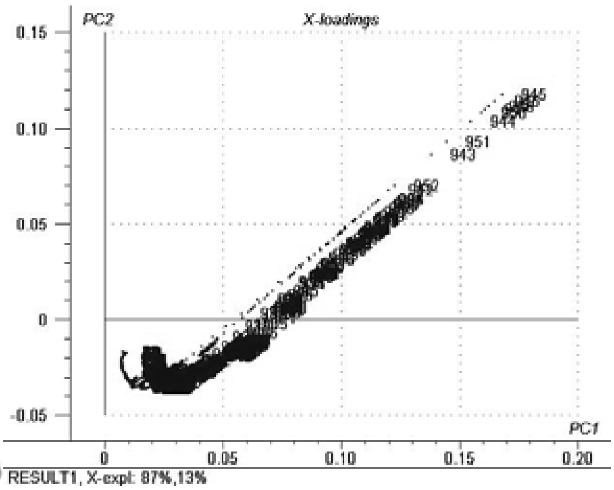


Fig. 2 Principal component analysis (PCA) of spectral reflectance

General speaking, our study may provide spectral index to estimate leaf chlorophyll fluorescence in field level. However, the literature reviewed on the detection of O_3-induced stress using traditional remote sensing techniques indicates that results were limited to the identification of the stress only in the damage phase. In fact, spectral variations were mostly identified at the end of fumigation period when visible symptoms already occurred [20]. Moreover, we are also aware that this analysis is the first step toward the goal of using remote sensing technology to monitor chlorophyll fluorescence in landscape level. The spectral response properties of vegetation canopy also have been found to depend on atmospheric (e.g., illumination, cloudy shadow), edaphic (e.g., soil type, soil moisture), and biotic (e.g., crop variety, leaf area index) conditions [21]. Thus, more studies are still needed by using airborne or satellite imagines in the future researches.

Table 2. Summary of the spectral indices used in this study

Spectral index	Category	References	Note
PSSRa: R_{800}/R_{680}	SR	[13, 14]	SR: Simple Ratios of reflectance or derivatives;
PSNDa: $(R_{800}-R_{680})/(R_{800}+R_{680})$	ND		
$(R_{780}-R_{710})/(R_{780}-R_{680})$	mSR	[15]	mSR: modified Simple Ratios of reflectance or derivatives;
SIPI: $(R_{800}-R_{445})/(R_{800}-R_{680})$	mSR	[16]	
$(R_{850}-R_{710}/(R_{850}-R_{680})$	mSR	[17]	ND: Normalized Difference of reflectance or derivatives.
NDSI(680,935): $(R_{680}-R_{935})/(R_{680}+R_{935})$	ND	[18]	
RSI(680,935): R_{680}/R_{935}	SR		

Table 3. Correlation coefficient (R) and root mean square error (RMSE) between spectral indices and chlorophyll fluorescence

Spectral index		Fo	Fm	Fv/Fm	Fv/Fo	ΦPSII	NPQ
PSSRa: R_{800}/R_{680}	R	-0.426*	0.501*	0.464*	0.571**	0.202	0.366
	RMSE	51.35	162.9	0.06	0.81	0.07	0.69
PSNDa: $(R_{800}-R_{680})/(R_{800}+R_{680})$	R	-0.417*	0.609**	0.481**	0.503*	0.253	0.302
	RMSE	52.30	184.9	0.04	0.79	0.07	0.64
$(R_{780}-R_{710})/(R_{780}-R_{680})$	R	-0.135	-0.102	-0.042	-0.055	0.450*	-0.677**
	RMSE	50.06	122.8	0.03	0.59	0.07	0.35
SIPI: $(R_{800}-R_{445})/(R_{800}-R_{680})$	R	-0.489*	-0.691**	-0.541**	-0.502*	0.517*	-0.018
	RMSE	53.61	211.6	0.05	0.25	0.10	0.91
$(R_{850}-R_{710}/(R_{850}-R_{680})$	R	0.179	-0.332	-0.317	-0.329	0.05	-0.499*
	RMSE	56.18	197.8	0.06	0.72	0.19	0.55
NDSI(680,935): $(R_{680}-R_{935})/(R_{680}+R_{935})$	R	0.793**	-0.751**	-0.818**	-0.815**	-0.551	0.138
	RMSE	41.06	128.0	0.03	0.52	0.08	0.62
RSI(680,935): R_{680}/R_{935}	R	0.813**	-0.716**	-0.729**	-0.826**	-0.502	0.122
	RMSE	49.25	130.9	0.03	0.49	0.07	0.59

Note: n=20, $P_{0.05}$=0.423, $P_{0.01}$=0.537

Acknowledgements

This research was supported by the Open Program of State Key Laboratory of Rice Biology (090402), National Natural Science Foundation of China (30900880, 30800126), and Forward Program of Key Laboratory of Digital Agriculture, ZAAS, China.

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