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Full Length Research Paper

Evaluating Absorption-Based Chlorophyll Indices for Accurate Prediction of Rice Leaf Chlorophyll Concentration

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Our objective in this study was to develop spectral absorption indices for prediction of leaf chlorophyll concentration based on blue/yellow/red/ edge absorption spectrum. Two field experiments were conducted to study the response of chlorophyll index based on leaf absorption spectra to chlorophyll concentration in rice. The ultimate, penultimate and third expanded leaves were sampled for chlorophyll measurements and the absorption spectra of the leaves on the main stem for three rice varieties at different growth stages to select the absorption wavelength position near zero and develop better algorithms for estimating chlorophyll concentration. Some indices called blue/yellow/red/ edge absorption spectra chlorophyll index (BEACI/ YEACI/ REACI) were calculated from elected absorption wavelength positions. For the 1st experiment the correlation coefficients were similar between chlorophyll concentration and single leaf spectral absorption and between chlorophyll concentration and these indices. But the chlorophyll concentration had significant correlations (P<0.01) to these indices than single leaf spectral absorption in the 2nd experiment. The liner regression models with single leaf spectral absorption $y = -2.271A_{480.188} + 5.574A_{651.232} - 2.899A_{753.552} - 0.269$, $y= -4.079A_{480.188} - 2.233A_{753.552} + 5.892A_{663.239} + 0.547$ and $y = 4.217A_{651.232} -0.718A_{753.552} - 2.897A_{663.239} - 0.399$ had higher power prediction total chlorophyll, chlororphyll a and chlorophyll b concentrations, respectively. Compared with BEACI and REACI, stepwise regression analysis showed that $YEACI_{630.610}$, $YEACI_{570.169}$ and $YEACI_{651.232}$ were good predictive power for predicting chlorophyll total concentration, chlorophyll a concentration and chlororphyll b concentration respectively.

Key words: Rice, chlorophyll concentration, leaf absorption spectrum, vegetation index.

INTRODCTION

Nondestructive determination of leaf chlorophyll content permits the measurements of changes in pigments over time for leaves and avoids time-consuming and expen-sive traditional chlorophyll concentration measurements (Gao et al., 2008). Spectral bands in the visible and near-infrared regions of the spectrum have been used to deve-lop a number of indices for estimating chlorophyll content. Vegetation index is a simple, effective and experiential measurement of terrestrial vegetation activity, and plays a very important role in qualitative and quantitative remote sensing. For remote sensing purposes, almost all indices based on reflectance spectrum including single band spectral reflectance and reflectance band ratios or diffe-rences have been used as indicators of chlorophyll con-tent of leaves. Various vegetation indices (VIs) have been related to chlorophyll content. The ratio vegetation index (RVI; Pearson and Miller, 1972) and the normalized diffe-rence vegetation index (NDVI; Rouse et al., 1974) are the best-known indices. NDVI is defined as (reflectance of NIR-reflectance of RED)/ (reflectance of NIR+ reflectance of RED). Some studies have developed RVI (Bisun, 1998; Guli, 2007). For example Andrea M et al. (2001) re-ported the best indices for Chl, Chl-a and Chl-b determi-nation of four different plants leaves were R′542/R′750, R′706/R′750 and R′556/R′ 750, respectively. Some spec-tral indices were reported such as the transformed chlo-rophyll absorption in reflectance index (TCARI; Kim et al.,

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1994) , the modified chlorophyll absorption in reflectance index (MCARI; Daughtry et al., 2000), the modified chlorophyll absorption continuum index (MCACI; Yang et al., 2006) and so on. By developing a new spectral index that reduces the effect of differences in leaf surface reflectance, Sima et al. (2002) were able to significantly improve the correlations with chlorophyll content. Their results demonstrate that spectral indices can be applied across species with widely varying leaf structure without the necessity for extensive calibration for each species. Cheng (2003) showed that the most suitable estimated model of chlorophyll a of upper leaves was obtained by using some hyper-spectral variables such as SDr, SDb and their integration. Anatoly AG (2003) reported that reciprocal reflectance (R)$^{-1}$ in the spectral range from 520 to 550 nm and 695 to 705 nm related closely to the total chlorophyll content in leaves of all studied species. Subtraction of near infra-red reciprocal reflectance, $(R_{NIR})^{-1}$, from (R)$^{-1}$ made index [(R)$^{-1}$-$(R_{NIR})^{-1}$] linearly proportional to the total chlorophyll content in spectral ranges from 525 to 555nm and from 695 to 725 nm with coefficient of determination $r^2 > 0.94$.The continuum in-dex using the spectral continuum on which the analyses are based on the area of the troughs spanned by the spectral continuum were reported (Zhang JH 2006). The position of the inflexion point in the red edge region (680 to 780 nm) of the spectral reflectance signature, termed the red edge position (REP), was affected by biochemical and biophysical parameters and had been used as a means to estimate foliar chlorophyll or nitrogen content. Many studies have focused on estimation of chlorophyll concentration using red edge characteristics. The red-edge, centered at the largest change in reflectance per wavelength change, is located between the red trough and the NIR plateau. Strong correlations have been found between the red edge and the chlorophyll concentration of leaves or canopy (Pinar1996). Some studies reported that among red edge parameters (such as $_{red}$; Min $_{600-720}$;d ; d $_{min}$; d $_{red}$ / d $_{min}$; d $_{680-750}$ and $_{nir}$) , the $_{red}$ can be used to estimate chlorophyll content satisfactorily, Zhao, 2002; Tang, 1996; 2004; Seager, 2005).

Dash (2007) reported a new index called the MERIS terrestrial chlorophyll index (MTCI) (MERIS denotations the medium resolution imaging spectrometer, has fine spectral resolution, moderate spatial resolution and a 3-day repeat cycle). MTCI uses data in three red/NIR wave-bands centered at 681.25, 708.75 and 753.75 nm, which lie in red edge range. Preliminary indirect evaluation us-ing model, field and MERIS data suggested its sensitivity to chlorophyll content, notably at high values (Dash, 2007). Spectroscopy can provide information about a substance by relating the interaction of electromagnetic radiation as a function of wavelength to its chemical com-position and physical properties. All vegetation contains the same basic constituents: chlorophyll and other light- absorbing pigments, water, proteins, starches, waxes, and structural biochemical molecules such as lignin and cellulose (Elvi-dge, 1990). All of these components contribute to the reflectance, transmissivity and absorption spectra of vegetation.

Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green. All photosynthetic organisms have chlorophyll a. Chlorophyll a absorbs its energy from the violet-blue and reddish orange-red wavelengths, and little from the intermediate (green-yellow-orange) wavelengths. Due to chlorophyll absorptions, the visible region of green plants shows a maximum reflectance at approximately 550 nm and lower reflectance in the blue (450 nm) and red (680 nm) (Pu, 2002). Green plants spectral curve from blue to green and from green to red called “blue edge” and “yellow edge” respectively. So chlorophyll concentration will affect green plants visible light spectral curve, which is response for the change of “blue edge” and “yellow edge” in visible light region. However, most of the vegetation indices or algorithms reported in the literature have not been developed using “blue edge” and “yellow edge”, and fewer studies predict chlorophyll concentration by the absorption spectrum from fresh leaves. Reported investigations about absorption spectrum prediction chlorophyll concentration including chlorophyll a, b and a+b pay attention to the absorption of the extracts at some wavelengths with spectrophotometer.

The main aims of this study were to determine blue, yellow and red edge positions to develop new models or vegetation indices for predicting chlorophyll concentration using fresh leaf absorption.

MATERIALS AND METHODS

Experiment description

The first field experiment was laid out in a split plot design with three replications. The main plots were arranged in split plot design block and comprised six basal nitrogen (N) rates: 0, 45,105, 165, 225, 300 kg N ha^{-1}. Subplots were three varieties of rice (*Oryza sa-tiva L*) Shengdao 13, Lindao11 and Yangguang 200, respectively with the similar growth stages. Main plot sizes were 6.0 × 4.0 m^2. The second experiment was conducted in a ploughed field with two varieties of rice (Yangguang200 and Lindao11). The field was divi-ded into four N supply areas as follows: 0 kg urea per hectare; 270 kg urea per hectare; 585 kg urea per hectare; 750 kg urea per hectare. For two field experiments, 50% N fertilizer was applied before trans-planting, 40% nitrogen fertilizer at tillering and 10% at heading, res-pectively.

Leaf absorption spectrum measurements and plant sampling

Twenty clumps of rice were obtained from each main plot at til-lering, booting and heading stage in the two experiments, and leaf absorption spectrum of the 1st, 2nd and 3rd expanded leaves were measured between 350-1100 nm in spectral resolution 2.4 nm by an AvaSpec -2048FT-SPU with light source of AvaLight-HAL. Their leaves were taken serving as subsamples being detached to measure chlorophyll a, chlorophyll b and total chlorophyll concentra-tions, respectively

Determination of chlorophyll concentrations in leaves

The fresh leaf mass was determined for the leaf samples prior to

Table 1. Statistics for chlorophyll a, chlorophyll b, and total chlorophyll concentrations.

		N	Mean	Range	Standard Deviation	Coefficient of Variation (%)
The 1st field experiment	Chl-a	271	1.882	0.742-3.075	0.396	21
	Chl-b	271	0.791	0.638-1.835	0.285	36
	Chl-t	271	2.673	0.972-4.084	0.572	21
The 2nd field experiment	Chl-a	95	2.030	1.275-2.783	0.37770	19
	Chl-b	95	0.811	0.477-1.221	0.16410	20
	Chl-t	95	2.841	1.807-3.908	0.51134	18

chlorophyll measurement. The 1st, 2nd and 3rd expanded leaves of each N supply level were weighed respectively to obtain the leaf mass. The chlorophyll was extracted in 80% acetone. The absorption of the extracts at wavelengths of 663nm (D_{663}) and 645 nm (D_{645}) were measured with a SP 722E spectrophotometer. The concentrations of chlorophyll a (Chl-a), chlorophyll b (Chl-b), and total chlorophyll (Chl-t) were then calculated using the equations as follow (Arnon, 1949).

$$\text{Chl-a} = 12.72A_{663}-2.59A_{645}$$
$$\text{Chl-b} = 22.9A_{645} -4.67A_{663}$$
$$\text{Chl-t} = 20.31\ A_{645} +8.05\ A_{663}$$

Statistical methods

All statistical analyses were conducted using the SPSS statistical software and $p < 0.05$ was used to determine significance in all tests. A number of statistics such as the mean, range, and standard deviation were used to describe the distribution of leaf absorption and chlorophyll data. The pearson correlation with 2-tailed signifi-cance tests were used to characterize the relationship between chlorophyll concentrations and vegetation indices, and the coeffi-cient of variation was also calculated as the ratio of the standard deviation to the mean. Multiple stepwise regressions were used to build and assess the chlorophyll prediction models with vegetation indices.

RESULTS AND DISCUSSION

Leaf chlorophyll concentration

The data on chlorophyll concentration showed a large range of chlorophyll concentrations. The chlorophyll a concentration lied between 70.41% for the 1st field experi-ment and 71.45% for the 2nd field experiment, respec-tively (Table 1), which consisted with some repoted con-clusions. Comar (1942) reported that chlorophyll a usual-ly lies between 67% and 78% for the normal green tis-sues of land higher plants, and formed much more rapidly than chlorophyll b. So prediction total chlorophyll and chlorophyll a concentration was higher than chlororphyll b. Gross (1991) reported that in higher plants, chlorophyll a was the major pigment and chlorophyll b was an acces-sory pigment, and the a/b ratio was generally around 3:1. And Lin (2002) reported that the chlorophyll a / chloro-phyll b ratio were generally around 3:2.

Selection characteristic wavelengths and develop-ment of vegetation indices

In order to develop better algorithms for estimating chlorophyll concentration, characteristic wavelengths were selected according to the peaks and dips of spectral absorption curves and the 1st derivative curve. The mean (n = 271) absorption spectra of the leaf samples of the first field experiment was showed in figure 1. The spectra of all samples were visually similar in shape and absorp-tions. Large variations in absorption magnitude could be observed in the blue, yellow and red edge position at the 1st derivative curve and correspond to the peaks and dips of spectral absorption curve (Figure 1), while wavelength positions were included in the Table 2.

Characteristics wavelengths were indicated by signifi-cant negative correlation coefficients and absorption of characteristics wavelengths were increased along with in-creased chlorophyll concentration. The correlations bet-ween chlorophyll concentrations and the characteristics wavelength positions were shown in Table 3. All reported correlation coefficients were significant at $P < 0.01$ except for $A_{753.552}$ (Table 3).

Three edge position chlorophyll indices were designed to estimate of chlorophyll concentration and was calcula-ted using the ratio of the difference in absorption among blue, yellow and red edge position. The blue/yellow/red/ edge absorption spectra chlorophyll index (BEACI/ YEACI/REACI) standard bands were selected according to Table 2, Table 3 and Figure 2. The following equations were developed for the estimation of chlorophyll a, b and total chlorophyll concentrations:

$$BEACI = \frac{A_{509.891} - A_{480.188}}{A_{550.507} - A_{509.891}} \quad (1)$$

$$REACI = \frac{A_{753.552} - A_{690.045}}{A_{690.045} - A_{676.368}} \quad (2)$$

$$YEACI = \frac{A_{676.368} - A_{x_i}}{A_{x_i} - A_{550.507}} \quad (3)$$

Where A_{x_i} indicates absorption of 663.239 nm (x_1), 651.232 nm (x_2), 645.509 nm (x_3), 630.610 nm (x_4), 610.510 nm (x_5), 600.731 nm (x_6) and 570.169 nm (x_7), respectively.

The relationship between chlorophyll a, b and total con-centrations and these indices are shown in Table 4. Com-

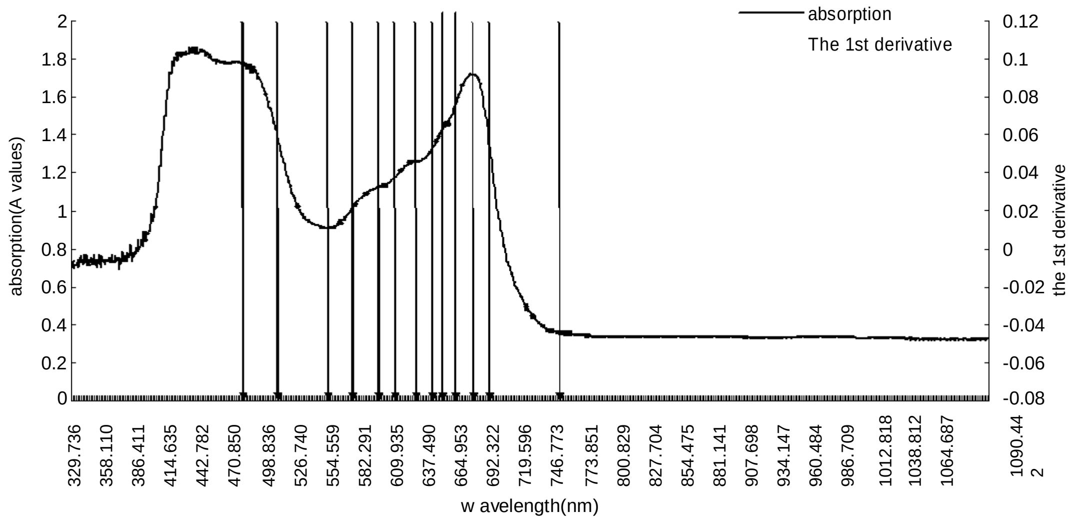

Figure 1. Selection charactistics wavelengths from spectral absorption scurve and its 1st derivative scurve.

Table 2. Wavelength positions were selected from Figure 1.

Spectral regions (nm)				
Blue edge	Yellow edge		Red edge	
480.188	570.169	600.731	690.045	753.552
509.891	610.510	630.610		
		550.507		
		676.3679		
		645.509		
		651.232		

663.239

pared with the relationship between single band spectra absorption and chlorophyll concentrations, the correlation coefficients increased significantly between chlorophyll concentrations and these indices with significant correlation at $P < 0.01$ (Tables 3 and 4).

Negative correlations were found between chlorophyll concentration and BEACI, and between chlorophyll concentration and REACI. As for YEACI, positive correlations were found with chlorophyll concentration.

The correlation coefficients between these indices and total chlorophyll concentrations were higher than these indices and concentrations of chlorophyll a or b. Therefore, based on the strong correlation, these indices could be used to predict chlorophyll concentrations especially total chlorophyll concentrations.

Chlorophyll prediction models

The relationship between these single band absorption and chlorophyll concentration were best described by multiple stepwise regression models (Table 5). Multiple stepwise regression models obtained between leaf absor-ption at different wavelengths (model-1: $A_{480.188}$, $A_{651.232}$, and $A_{753.552}$;model-2: $A_{480.188}$,$A_{753.552}$, and $A_{663.239}$; model-3:$A_{651.232}$, $A_{753.552}$,and $A_{663.239}$) and chlorophyll concentra-tions were highly correlated, suggesting that these mo-dels were better predictors to estimate total chlorophyll, chlorophyll a and chlorophyll b concentration respectively (Table 5).

The relationship between blue/yellow/red/ edge absor-ption spectra chlorophyll index (BEACI/YEACI/REACI) and chlorophyll concentrations were best described by liner regression models. Liner regression models obtained between chlorophyll concentrations and blue/red edge absorption spectra chlorophyll index (BEACI, REACI) had similar correlation coefficients, suggesting that BEACI and REACI had similar perdition power to predict chloro-phyll concentrations. However, compare with BEACI, REACI and single band models (model-1, model-2 and model-3), regression analysis indicated that better predic-tive liner regression models with $YEACI_{630.610}$, $YEACI_{600.731}$ and $YEACI_{651.232}$ for predicting Chl-t, Chl-a and Chl-b concentrations with the higher R^2 (0.740, 0.656 and 0.620) respectively (Table 6).

Validation

The models described in Table 5 and algorithm described

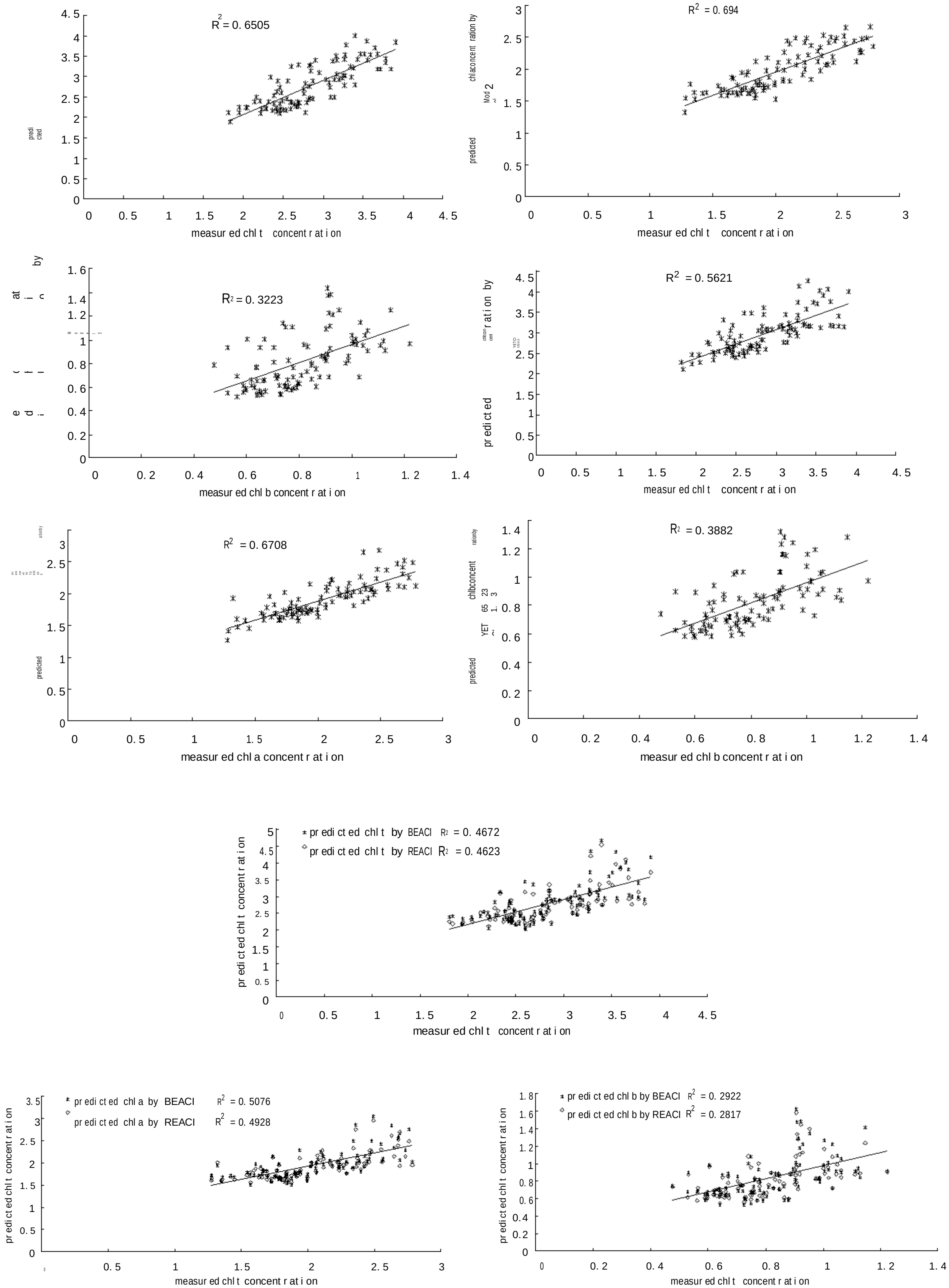

Figure 2. The relationship between the predicted and measured values for testing power of prediction models.

Table 3. The correlation coefficients between chlorophyll concentrations (Chl- a, Chl-b and Chl-t) and vegetation indices. (n=273.* and ** indicated that correlation is significant at the 0.05 level and 0.01 level respectively. A indicated absorption of waveband.)

	The 1st experiment			The 2nd experiment		
	Chl-a	**Chl-b**	**Chl-t**	**Chl-a**	**Chl-b**	**Chl-t**
$A_{480.188}$	0.596(**)	0.605(**)	0.713(**)	0.628(**)	0.290(**)	0.576(**)
$A_{509.891}$	0.600(**)	0.618(**)	0.722(**)	0.637(**)	0.288(**)	0.581(**)
$A_{550.507}$	0.542(**)	0.586(**)	0.667(**)	0.560(**)	0.204(*)	0.493(**)
$A_{570.169}$	0.563(**)	0.593(**)	0.684(**)	0.589(**)	0.234(*)	0.526(**)
$A_{600.731}$	0.586(**)	0.605(**)	0.706(**)	0.624(**)	0.272(**)	0.566(**)
$A_{610.510}$	0.592(**)	0.608(**)	0.712(**)	0.633(**)	0.281(**)	0.575(**)
$A_{630.610}$	0.601(**)	0.613(**)	0.720(**)	0.646(**)	0.295(**)	0.590(**)
$A_{645.509}$	0.609(**)	0.617(**)	0.728(**)	0.665(**)	0.319(**)	0.614(**)
$A_{651.232}$	0.613(**)	0.619(**)	0.732(**)	0.673(**)	0.328(**)	0.623(**)
$A_{663.239}$	0.617(**)	0.608(**)	0.729(**)	0.682(**)	0.337(**)	0.633(**)
$A_{676.368}$	0.604(**)	0.589(**)	0.711(**)	0.666(**)	0.326(**)	0.617(**)
$A_{690.045}$	0.603(**)	0.613(**)	0.722(**)	0.654(**)	0.306(**)	0.601(**)
$A_{753.552}$	0.115	0.256(**)	0.207(**)	0.156	-0.161	0.058

Table 4. The correlation between chlorophyll concentrations (Chl-a, Chl-b and Chl-t) and vegetation indices. (.* and ** (n = 273) indicate that correlation is significant at the 0.05 level and 0.01 level respectively).

	Chl-a	**Chl-b**	**Chl-t**
BEACI	0.607(**)	0.594(**)	0.715(**)
REACI	0.593(**)	0.608(**)	0.713(**)
$YEACI_{x1}$	-0.626(**)	-0.604(**)	-0.733(**)
$YEACI_{x2}$	-0.607(**)	-0.620(**)	-0.728(**)
$YEACI_{x3}$	-0.611(**)	-0.615(**)	-0.729(**)
$YEACI_{x4}$	-0.627(**)	-0.616(**)	-0.740(**)
$YEACI_{x5}$	-0.631(**)	-0.604(**)	-0.736(**)
$YEACI_{x6}$	-0.637(**)	-0.595(**)	-0.736(**)
$YEACI_{x7}$	-0.656(**)	-0.543(**)	-0.723(**)

Table 5. Chlorophyll prediction models using single band absorption.

Dependent variable	**Model**	**R^2**	**F**	**Sig.**
Chl-t	Model1 $y=-2.271A_{480.188}+5.574A_{651.232}-2.899A_{753.552}-0.269$	0.625	150.011	0.00
Chl-a	Model2 $y=-4.079A_{480.188}-2.233A_{753.552}+5.892A_{663.239}+0.547$	0.504	91.593	0.00
Chl-b	Model3 $y=4.217A_{651.232}-0.718A_{753.552}-2.897A_{663.239}-0.399$	0.417	64.352	0.00

Table 6. Chlorophyll prediction models with these indices. (n = 273).

Dependent variable	**Predictor**	**Model**	**R**	**R^2**	**F**	**Sig.**
Chl-t	$YEACI_{630.610}$	Y = -3.027x+6.801	0.740	0.547	328.766	0.00
	BEACI	Y = -0.877+3.081x	0.715	0.512	284.995	0.00
	REACI	Y = 0.824x+0.084	0.713	0.508	281.070	0.00
Chl-a	$YEACI_{600.731}$	Y = -0.286x+4.662	0.656	0.430	205.042	0.00
	BEACI	Y = 1.8025x - 0.1963	0.613	0.376	161.970	0.00
	REACI	Y = 0.473x +0.395	0.593	0.352	147.746	0.00
Chl-b	$YEACI_{651.232}$	Y = -2.258x+2.025	0.620	0.384	169.641	0.00
	BEACI	Y = 1.279x-0.681	0.594	0.353	148.283	0.00
	REACI	Y = 0.351x-0.311	0.608	0.370	159.587	0.00

in Esq (1)-(3) was tested using a validation data set(n = 95) of the second experiment. The absorption measurements of the validation samples were used to calculate the $YEACI_{630.610}$, $YEACI_{600.731}$, $YEACI_{651.232}$, BEACI and REACI, and then the models and algorithm equations were used to predict the chlorophyll concentrations. The highly significant correlation between the predicted and measured values was indicated by the scatter -plots (Fi-gure 5). High positive correlations between the predicted chlorophyll concentrations and actual chlorophyll concen-trations showed good predictive power of predicting chlo-rophyll total concentration using model1and $YEACI_{630.610}$ with the higher R^2(0.6505,0.5621), predicting chlorophyll a concentrations using model2 and $YEACI_{600.731}$ with the higher R^2(0.694,0.6708). Good predictive power of predicting Chl-a and Chl-t using BEACI and REACI were reported with higher R^2 (Figure 2).

Conclusion

Analysis of visible/near-infrared absorption spectrum and chlorophyll data for a wide range of rice leaves has developed better algorithms for the quantification of chloro-phyll concentration by spectral absorption. It was found that five vegetation indices (BEACI, REACI, $YEACI_{630.610}$, $YEACI_{600.731}$, and $YEACI_{651.232}$) were reported higher significant correlation with chlorophyll concentration. Three better chlorophyll concentration predictors were found as follow: $YEACI_{630.610}$ predicting chlorophyll total concentration; $YEACI_{600.731}$ predicting chlorophyll a concentration; $YEACI_{651.232}$ predicting chlorophyll b concentration. From single band spectral absorption, three models (chlt = - 2.271 $A_{480.188}$+ 5.574$A_{651.232}$- 2.899$A_{753.552}$- 0.269; chla = - 4.079$A_{480.188}$ -2.233$A_{753.552}$+ 5.892$A_{663.239}$ +0.547; chlb = 4.217$A_{651.232}$ -0.718$A_{753.552}$ -2.897$A_{663.239}$ - 0.399) were reported having good predictive power with higher correlation between predicted and measured chlorophyll concentrations. This study indicated that satisfactory re-sults have been obtained to predict chlorophyll concen-trations using selected characteristic wavelengths and developed vegetation indices.

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