



Estimate Leaf Chlorophyll of Rice Using Reflectance Indices and Partial Least Squares

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Summary: In this study field experiments were conducted to test the ability of optimized spectral indices and partial least squares (PLS) to estimate leaf chlorophyll (Chl) content of rice from non-destructive canopy reflectance measurements. We integrated techniques involving the optimization of narrow band spectral indices and the detection of red edge position to optimize one type of spectral indices, the *ratio of reflectance difference index* (RRDI), for the estimation of leaf Chl content. The optimized RRDI in the *red-edge* ($RRDI_{re} = (R_{745} - R_{740}) / (R_{740} - R_{700})$) accounted for 62% – 72% of the variation in leaf Chl content with an RMSE of $4.59 \mu\text{g}/\text{cm}^2 - 4.89 \mu\text{g}/\text{cm}^2$. Compared to spectral indices, PLS improved the estimation of leaf Chl content, yielding R^2 and RMSE of $0.85 \mu\text{g}/\text{cm}^2$ and $3.22 \mu\text{g}/\text{cm}^2$, respectively. Finally, the model based on RRDI and the PLS model were further validated by an independent dataset collected in farmer fields. RRDI and PLS models yielded acceptable accuracy with R^2 of 0.49 and 0.55, respectively, and an RMSE of $5.47 \mu\text{g}/\text{cm}^2$ and $5.13 \mu\text{g}/\text{cm}^2$. Our results suggest the potential to optimize spectral indices and also the significance of PLS technique for mapping canopy biochemical variations.

Zusammenfassung: *Abschätzung von Blatt-Chlorophyllgehalten von Reis mit Hilfe von Spektralindizes und Partial Least Squares Analysen.* In dieser Studie wurden Feldversuche durchgeführt, um optimierte Spektralindizes und Partial Least Squares (PLS) Analysen für die Abschätzung von Blatt-Chlorophyllgehalten von Reis mittels nicht destruktiven Reflexionsmessungen zu evaluieren. Es wurden unterschiedliche Analysemethoden von hyperspektralen Daten integriert. Ziel der Untersuchung ist die Optimierung eines bestimmten Typs von spektralen Indizes, den *Ratio of Reflectance Difference Index* (RRDI). Letzterer wurde für die Abschätzung von Blatt-Chlorophyllgehalten entwickelt. Der optimierte RRDI im *red-edge* ($RRDI_{re} = (R_{745} - R_{740}) / (R_{740} - R_{700})$) erklärte 62% – 72% von der Variabilität des Blatt-Chlorophyllgehalts mit einem RMSE von $4.59 \mu\text{g}/\text{cm}^2 - 4.89 \mu\text{g}/\text{cm}^2$. Im Vergleich zu etablierten Spektralindizes kann mittels der PLS Analyse die Abschätzung von Blatt-Chlorophyllgehalten signifikant verbessert werden ($R^2 = 0.85$, $RMSE = 3.22 \mu\text{g}/\text{cm}^2$). Schließlich wurden die RRDI- und PLS-basierten Modelle anhand eines unabhängigen Datensatzes, der auf landwirtschaftlich genutzten Feldern erhoben wurde, zusätzlich validiert. Die RRDI- und PLS-Modelle erzielten eine akzeptable Genauigkeit mit jeweils einem R^2 von 0.49 und 0.55 und einem RMSE von $5.47 \mu\text{g}/\text{cm}^2$ und $5.13 \mu\text{g}/\text{cm}^2$. Unsere Ergebnisse unterstreichen das Potential für die Optimierung von Spektralindizes sowie die Bedeutung von PLS Analysen für die Bestandskartierung von biochemischen Variationen.

1 Introduction

Rice is one of the main agricultural crops in Northeast China. The Sanjiang Plain is well known for large scale farming in China and

is becoming more and more important in supplying the food market with commercial rice of high quality (YAO et al. 2012). For a high-yield and environment-friendly agricultur-

al development, real-time monitoring of the growth status of rice is crucial to this region.

Remote sensing is increasingly being used in agricultural applications owing to its potential for the noninvasively gathering of information over larger areas (ATZBERGER 2013, MULLA 2013). Hyperspectral remote sensing of crop nutrient status is mainly based on the estimation of leaf chlorophyll (Chl), which absorbs and converts solar light to biochemical energy and thus often serves as an indicator of plant stresses (FILELLA & PEÑUELAS 1994). Recent studies have shown great potential of hyperspectral remote sensing for the estimation of leaf chlorophyll (ZARCO-TEJADA et al. 2001), plant nitrogen (N) (YU et al. 2013), leaf area index (LAI) (DARVISHZADEH et al. 2009), biomass (GNYP et al. 2013, GNYP et al. 2014, KOPPE et al. 2012) and for disease detection (DELA-LIEUX et al. 2009, LAUDIEN et al. 2006, LAUDIEN & BARETH 2006).

The red edge (ca. 680 nm – 750 nm) of spectra is of particular interest for estimating leaf chlorophyll content (FILELLA & PEÑUELAS 1994, MAIN et al. 2011). The red edge position (λ_{RE}) is defined as the wavelength of the peak (local maximum) on the first derivative reflectance spectra (HORLER et al. 1983). Generally, λ_{RE} shifts to longer wavelengths with the increase of Chl content (FILELLA & PEÑUELAS 1994). Several studies have found that two or more peaks in red edge can be derived from the derivative spectra (HORLER et al. 1983, ZARCO-TEJADA et al. 2002). HORLER et al. (1983) suggested that the first peak at around 700 nm is determined mainly by chlorophyll content while the second peak at around 725 nm is governed more by scattering effects.

Recent studies have shown that optimized narrow band spectral indices perform better than broad band indices for the estimation of Chl and LAI (DARVISHZADEH et al. 2008, DARVISHZADEH et al. 2009). In most of these studies narrow band indices take the forms of simple ratio (SR) and normalized difference vegetation index (NDVI) to find the best band combinations. YU et al. (2012) found that the optimized SR- and NDVI-like indices have similar sensitive bands and provide equal ability to estimate Chl.

The objective of this study is to test the ability of new spectral indices and the partial least

squares (PLS) method to estimate the leaf Chl content of rice.

2 Material and Methods

2.1 Study Area, Experimental and Farmer Fields

The study area is located in the Sanjiang Plain, Heilongjiang Province, China. The Sanjiang Plain was originally dominated by marshes and it was converted to agricultural production six decades ago (YAO et al. 2012). The climate in this region is cool-temperate subhumid continental monsoon, with very cold winters and warm summers. The climatic characteristics of Sanjiang Plain are suitable for rice, soybeans, wheat, and corn crops. Rice farming has become the dominant land use in this region in the last two decades. More information about the Sanjiang Plain has been provided elsewhere (GNYP et al. 2013, YAO et al. 2012, YU et al. 2013). In this study, two field experiments (Exp. 1 and Exp. 2) were conducted, and 14 farmer fields were selected for data collection.

Exp. 1: The N rate experiment was conducted at two sites: Qixing and Keyansuo experimental stations with a same experimental design in 2008. A randomized complete block design with four replications including five N rates (0, 35, 70, 105 and 140 kg N ha⁻¹ as urea, CO(NH₂)₂) was applied at both stations, where a local rice cultivar *Kongyu131* was planted. 60 kg ha⁻¹ P₂O₅ (as triple super-phosphate) and 75 kg ha⁻¹ K₂O (as potassium sulfate) were applied to ensure the supply of other nutrients. All plots had the same size of 100 m² (10 m by 10 m).

Exp. 2: Similar design with Exp. 1, Exp. 2 was conducted under five N levels that used 70% of each of the five rates of Exp. 1, which was 0, 24.5, 49, 73.5 and 98 kg N ha⁻¹, respectively. The same cultivar *Kongyu131* and same amount of P- and K-fertilizers were used.

Farmer fields: In addition to the experimental fields, 14 farmer fields managed by two farmers were selected for data collection, which is to be used as the validation dataset. Farmers applied fertilizers according to their own experiences and local practices. The

same cultivar *Kongyu131* was planted in those farmer fields.

2.2 Spectral Measurement

Hyperspectral reflectance data was measured from a height of 30 cm above the rice canopy under clear sky conditions within 2 hours of solar noon, using the FieldSpec 3 spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO, USA) connected to a fiber fore-optic that has a 25 degree field-of-view. The FieldSpec 3 spectroradiometer operates in the 350 nm – 2500 nm spectral region and has a spectral resolution of 3 nm at 700 nm, 10 nm at 1400 nm and 2100 nm. The detailed description of FieldSpec 3 can be found in GNYP et al. (2013). Hyperspectral reflectance data in 1 nm steps were automatically output by the spectroradiometer. We used the reflectance data of 350 nm – 900 nm in this study due to the specific interest in Chl.

2.3 Leaf Chlorophyll Measurement

On the same day of spectral measurements, leaf chlorophyll was measured using a SPAD-502 (Konica Minolta, Inc.) chlorophyll meter. In those spectroradiometer-scanned plants, a total of 10 – 15 newly, fully expanded leaves were selected for recording SPAD values. For each leaf, 3 replicates were recorded in the middle position of the leaf base to tip and then averaged. Finally, SPAD values were transformed to area-based leaf chlorophyll content (Chl, $\mu\text{g}/\text{cm}^2$) using an empirically calibrated function commonly used in remote sensing studies (ATZBERGER et al. 2003, DARVISHZADEH et al. 2008, MARKWELL et al. 1995).

2.4 Reflectance Indices

An NDVI-like index, the normalized reflectance difference index (NRDI, (1)), was optimized using a lambda-by-lambda band optimization (LLBO) method, which has been widely used in recent studies (DARVISHZADEH et al. 2008, DARVISHZADEH et al. 2009, YU et al. 2013).

$$NRDI = \frac{R_{\lambda 1} - R_{\lambda 2}}{R_{\lambda 1} + R_{\lambda 2}} \quad (1)$$

where R_{λ} is the reflectance at the wavelength λ . The LLBO method thoroughly examines all the possible pairs of the bands $\lambda 1$ and $\lambda 2$ for NRDI for the correlation with the response variable of interest, chlorophyll in this study.

To test whether we can further improve the robustness of optimized indices, we made a hypothesis, which assumes that R_c is the reflectance in response primarily to chlorophyll and is a function of the wavelength λ , i.e., $R_c = f(\lambda)$. However, due to effects of soil, water background and phenological development, the measured canopy reflectance (R) can be further assumed as a function of R_c and the constants, a and b , that have multiplicative and additive factors respectively, across wavelengths (2),

$$R = a \cdot f(\lambda) + b \quad (2)$$

Although such a linear hypothesis is rare in nature, we expect that it allows for the removal of the adverse effects added to the canopy reflectance (R). R_c could be then calculated by eliminating the factors a and b from the measured reflectance R , following (3),

$$R_c = (R - b) / a \quad (3)$$

However, since a and b are difficult to determine, an alternative approach to eliminate a and b is to use the ratio of reflectance difference as shown in (4),

$$\frac{R_{c,\lambda 1} - R_{c,\lambda 2}}{R_{c,\lambda 3} - R_{c,\lambda 4}} = \frac{R_{\lambda 1} - R_{\lambda 2}}{R_{\lambda 3} - R_{\lambda 4}} \quad (4)$$

Finally, we define the ratio of reflectance difference index (RRDI, (5)) as:

$$RRDI = \frac{R_{\lambda 1} - R_{\lambda 2}}{R_{\lambda 3} - R_{\lambda 4}}, \quad (5)$$

for which $\lambda 1 - \lambda 4$ are random wavelengths to be optimized for the estimation of Chl. The RRDI optimization is achieved through two steps. First step, all possible 2-band combinations of $\lambda 1$ and $\lambda 2$ within the range of 350 nm

– 900 nm are examined for the correlation with Chl, for which the best correlation (highest R^2) produces the best NRDI. The best λ_1 and λ_2 determined in this step will be used as the numerator in RRDI. Second step, all possible band combinations of λ_3 and λ_4 are examined for the correlation with Chl, for which the best correlation produces the best RRDI.

2.5 PLS Model

The PLS method is an efficient tool for multivariate modeling and is increasingly used for handling high dimensional hyperspectral data (RICHTER et al. 2012). The PLS regression reduces the data dimension by extracting the latent variables (factors) as new predictors and regress the response variables on these factors. Compared to multiple linear regression, the PLS regression has the desirable property that solves the problem of strong co-linearity (ATZBERGER et al. 2010). Therefore, PLS was also used to estimate Chl in this study. PLS has the advantage that the precision of the model improves with the increasing number of variables and observations (WOLD et al. 2001). To optimize the number of factors (latent variables), leave-one-out cross valida-

tion was used to test the significance of the increase in the predicted residual sum of squares (PRESS) (VAN DER VOET 1994).

3 Results

3.1 NRDI Optimization

Fig. 1A shows the lambda-by-lambda R^2 plot for the correlations between NRDI and Chl. The highest R^2 values were obtained by the red edge bands paired with NIR bands.

Fig. 1B shows the best NRDI (highest R^2) with λ_1 and λ_2 at 745 nm and 740 nm, respectively. This NRDI accounted for 70% of the variation in Chl with an RMSE of 4.8 $\mu\text{g}/\text{cm}^2$ (Fig. 1B).

3.2 Red Edge Position

The red edge position (λ_{RE}) was determined as the maximum of the first derivative of the reflectance. Fig. 2 shows that λ_{RE} ranged from 700 nm to 740 nm and yielded significant difference only when N rate was higher than 105 kg/ha.

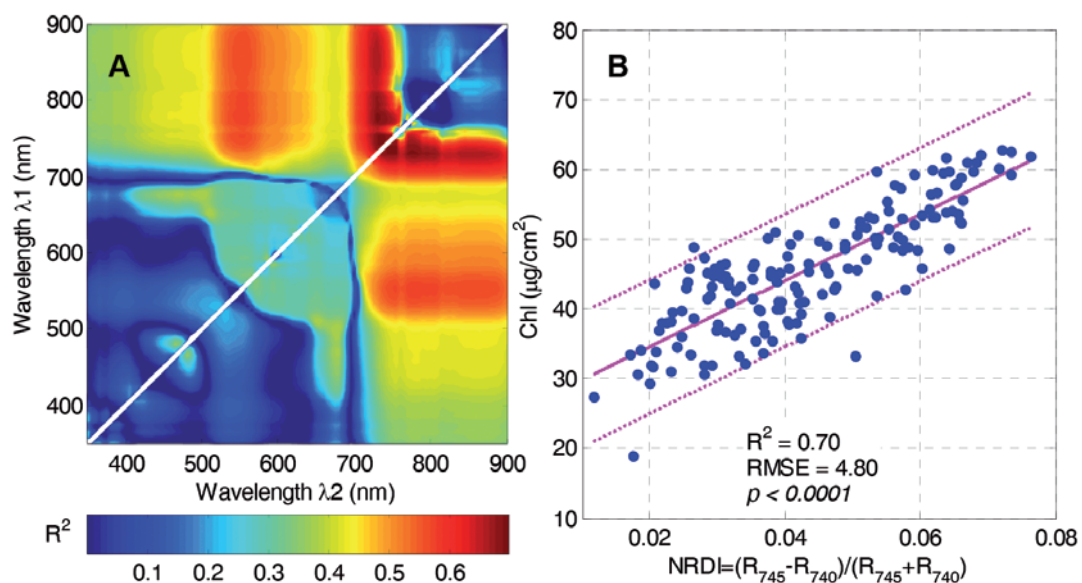


Fig. 1: A: Lambda-by-lambda R^2 plot showing the performance of different band combinations of λ_1 and λ_2 for the optimization of NRDI. B: Scatter plot showing the relationship between Chl and the best 2-band combination of λ_1 and λ_2 derived from Fig. 1A.

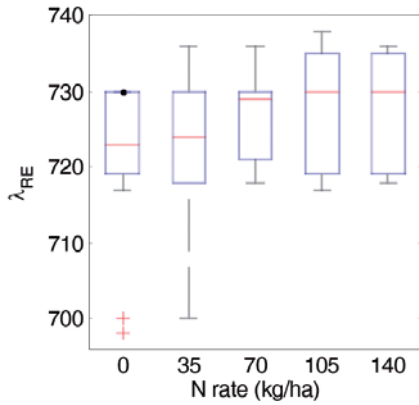


Fig. 2: Boxplot showing the changes in red edge position (λ_{RE}) between different N rates.

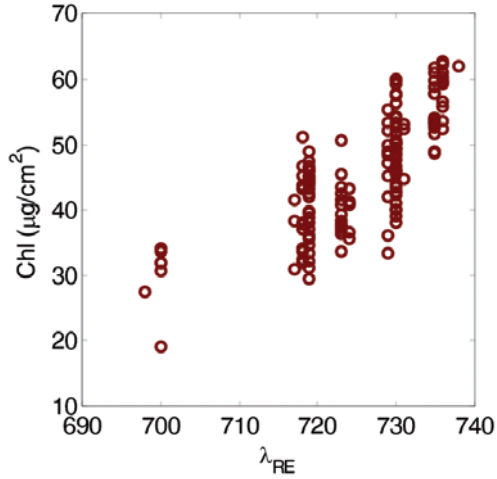


Fig. 3: Leaf chlorophyll content (Chl, $\mu\text{g}/\text{cm}^2$) plotted as a function of the red edge position (λ_{RE}).

The N rates of 105 kg/ha and 140 kg/ha produced a λ_{RE} shift to longer wavelengths and yielded higher values of λ_{RE} compared to the low-N rates.

To investigate the response of λ_{RE} to Chl variations, Chl was plotted as a function of λ_{RE} . Fig. 3 shows that λ_{RE} was positively related to Chl. The highest value of λ_{RE} , ca. 740 nm, corresponded to the highest Chl content that was $65 \mu\text{g}/\text{cm}^2$ approximately.

3.3 RRDI Optimization

Fig. 4 shows the lambda-by-lambda R^2 plot for the correlations between RRDI and Chl. Re-

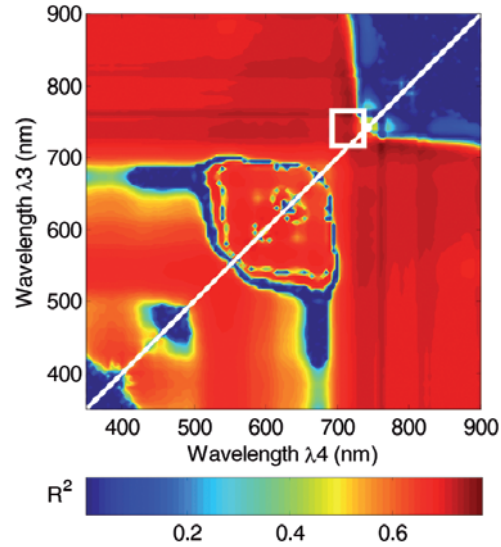


Fig. 4: Lambda-by-lambda R^2 plot showing the performance of different band combinations of λ_3 vs. λ_4 for the RRDI. White rectangle highlights the red edge range of 700 nm – 740 nm.

sults indicate that RRDI greatly increased the sensitivity to Chl across the whole wavelength range compared to NRDI (Fig. 1A).

One of the significantly hot zones for λ_3 vs. λ_4 locates in the wavelengths of 700 nm – 740 nm, which agrees well with the range of λ_{RE} (Figs. 2 and 3). Therefore, the λ_3 vs. λ_4 were optimized within the range of 700 nm – 740 nm and, they were finally determined as 740 nm and 700 nm based on the highest R^2 , respectively, for the best RRDI.

The best RRDI = $(R_{745} - R_{740}) / (R_{740} - R_{700})$ accounted for 72% of the variation in Chl with an RMSE of $4.59 \mu\text{g}/\text{cm}^2$ (Tab. 1).

Exp. 2 dataset was used to test the reliability of the best NRDI and RRDI for the estimation of Chl. Results show that NRDI and RRDI accounted for 60% and 62% of the variation in Chl, respectively, with an RMSE of $4.77 \mu\text{g}/\text{cm}^2$ and $4.63 \mu\text{g}/\text{cm}^2$ (Tab. 1: Results of R^2 and RMSE ($\mu\text{g}/\text{cm}^2$) for different datasets using NRDI = $(R_{745} - R_{740}) / (R_{745} + R_{740})$, RRDI = $(R_{745} - R_{740}) / (R_{740} - R_{700})$ and the PLS model).

3.4 Chl Estimation for Farmer Fields

Regression models based on RRDI and NRDI were calibrated using the pooled data of the

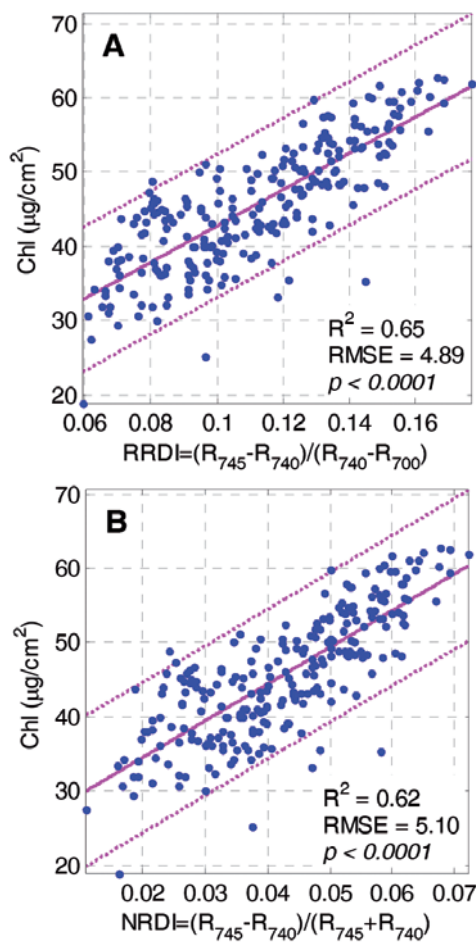


Fig. 5: (A) RRDI model based on the pooled experimental data (Exp. 1 + 2). (B) NRDI model based on the pooled data.

two experiments and were applied to farmer fields for the estimation of Chl.

Fig. 5 shows the calibration results for RRDI and NRDI. RRDI and NRDI accounted for 65% and 62% of the variation in Chl of pooled data (Exp.1 + 2), respectively.

Tab. 1 summarizes both the calibration and validation results for farmer fields. The R^2 for the predicted Chl by RRDI and NRDI against the measured Chl were 0.49 and 0.45, respectively, with an RMSE of 5.47 $\mu\text{g}/\text{cm}^2$ and 5.68 $\mu\text{g}/\text{cm}^2$ (Fig. 6A–B and Tab. 1).

3.5 Chl Estimation for Farmer Fields Using PLS model

The PLS model was also calibrated using the pooled data of two experiments. Results showed that PLS model accounted for 85% of the variation in Chl (Tab. 1) with an RMSE of 3.22 $\mu\text{g}/\text{cm}^2$.

The calibrated PLS model was further used to estimate the Chl of farmer fields. Fig. 6C shows that R^2 for the predicted Chl by PLS against the measured Chl was 0.55 with an RMSE of 5.13 $\mu\text{g}/\text{cm}^2$.

The PLS model accounted for a larger portion of the variation in Chl of both experimental and farmer fields and yielded a lower RMSE compared to the univariate regression models based on NRDI and RRDI (Tab. 1 and Fig. 6).

Tab. 1: Results of R^2 and RMSE ($\mu\text{g}/\text{cm}^2$) for different datasets using NRDI = $(R_{745} - R_{740}) / (R_{745} + R_{740})$, RRDI = $(R_{745} - R_{740}) / (R_{740} - R_{700})$ and the PLS model.

Dataset	Description	n	NRDI		RRDI		PLS	
			R^2	RMSE	R^2	RMSE	R^2	RMSE
Exp. 1	Optimize Indices	160	0.70	4.80	0.72	4.59		
Exp. 2	Test Indices	80	0.60	4.77	0.62	4.63		
Exp. 1 + 2	Model Calibration	240	0.62	5.10	0.65	4.89	0.85	3.22
Farmer fields	Model Validation	70	0.45	5.68	0.49	5.47	0.55	5.13

n , number of observations

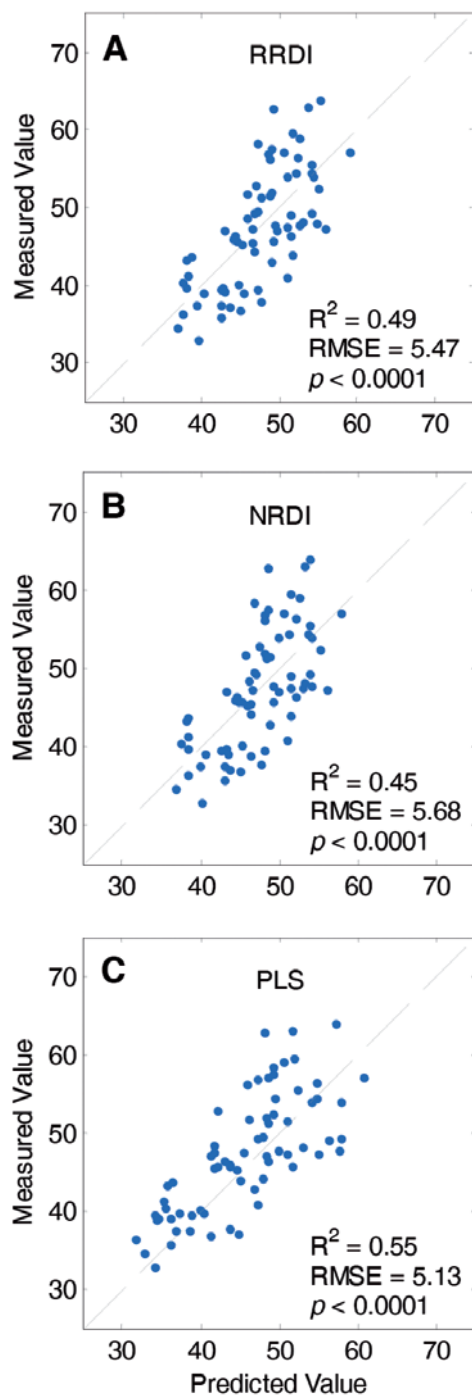


Fig. 6: Scatter plots showing the measured by predicted values of Chl using (A) RRDI, (B) NRDI and (C) PLS models calibrated on the pooled data of two experiments (Exp. 1 + 2). Dashed line is the 1:1 line.

4 Discussion

The lambda-by-lambda band optimization method has been used to optimize NDVI- and SR-like indices for the estimation of canopy characteristics in different species (DARVISHZADEH et al. 2008, DARVISHZADEH et al. 2009, YU et al. 2012, YU et al. 2013). However, the potential of linking red edge characteristics to the optimization of NDVI- or SR-like indices have not been fully explored. As shown in the lambda-by-lambda R^2 plots, the RRDI optimization increases the sensitivity over the entire wavelengths compared to NRDI (Figs. 1 and 4). In addition to the significant zone at red edge range, the range for NIR vs. red edge, e.g. 760 nm – 820 nm vs. 720 nm) also showed the best performance. However, the NIR range is governed primarily by LAI (DARVISHZADEH et al. 2008), thus the red edge might be more appropriate for Chl estimation.

The best $RRDI = (R_{745} - R_{740}) / (R_{740} - R_{700})$ could be considered as the ratio of derivative of reflectance at 740 nm, i.e. $d\lambda_{740} = (\lambda_{745} - \lambda_{740})/5$ and the relative change in the red edge positions. Similarly, LEE et al. (2008) found that the derivative of reflectance at 735 nm could be used to estimate rice N. Soil background is one of the main factors that affect the hyperspectral remote sensing of leaf chlorophyll. DARVISHZADEH et al. (2008) optimized the SAVI2 type indices to estimate Chl and found that it yielded equivalent accuracy in terms of RMSE compared to narrow band NDVI, i.e., NRDI in this study. However, the optimization of SAVI2 type indices requires the soil-line coefficients, which are difficult to determine for this study due to the flooding environment of rice field. Our results suggest that RRDI seems to be able to reduce to some extent the effects of soil, water background and phenological development compared to NRDI.

Spectral indices are not able to represent all spectral variability because they often employ a limiting number of bands. Also, the simple regression models based on spectral indices are easy to be over-fitted to the limiting observations. In contrast, PLS takes into account how the response variables co-vary with the explanatory variables, and it is particularly relevant in the situation where modeling data

consist of many predictors, i.e., hyperspectral narrow bands, relative to the number of observations (WOLD et al. 2001, Yu et al. 2014). As expected, PLS outperformed both the optimized NRDI and RRDI and resulted in lower RMSE. Both NRDI and RRDI showed an underestimation of high Chl values compared to the PLS model (Fig. 6). This corroborates the suggestion to use PLS for the full spectrum analysis (ATZBERGER et al. 2003). However, the determination of sensitive bands and optimization of spectral indices might be useful as an early indicator of plant physiological status and potential stresses before a more precisely quantitative approach made by full spectrum analysis.

Considering that spectral indices are characterized by simplicity and are compatible for different sensors with different resolutions or bands, the optimization of spectral indices still has practical value for applications of remote sensing in agriculture. Robust spectral indices will also contribute to the development of end-user-friendly crop sensors. Better development and validation of more complex, but more reliable, indices could be also achieved by integrating more rigorous cross-validation or bootstrap techniques (RICHTER et al. 2012).

5 Conclusions

The red edge plays a crucial role in estimating chlorophyll (Chl). This stresses the high potential of the red edge bands for the optimization of spectral indices. Two indices based on red edge: the normalized reflectance difference index ($NRDI = (R_{745} - R_{740}) / (R_{745} + R_{700})$) and the ratio of reflectance difference index ($RRDI = (R_{745} - R_{740}) / (R_{740} - R_{700})$) are robust indicators of leaf Chl content of rice ($R^2 = 0.60 - 0.72$, $RMSE = 4.59 \mu\text{g}/\text{cm}^2 - 5.1 \mu\text{g}/\text{cm}^2$) according to experimental data. They showed acceptable performance for mapping the Chl variation in agricultural fields, yielding an RMSE of $5.68 \mu\text{g}/\text{cm}^2$ and $5.47 \mu\text{g}/\text{cm}^2$, respectively, although the partial least squares (PLS) model delivered higher accuracy ($RMSE = 5.13 \mu\text{g}/\text{cm}^2$). The results show the potential of mapping canopy biochemical traits through

the optimization of spectral indices and other feature reduction techniques such as PLS.

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