NITROGEN ESTIMATION OF OILSEED RAPE USING SPAD AND VIS/NIR SPECTROSCOPY

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CSBE101397 – Presented at Section III: Equipment Engineering for Plant Production Conference

ABSTRACT The estimation of nitrogen status, non-destructively, in oilseed rape was performed using spectral reflectance with visible and near infrared reflectance spectroscopy (Vis/NIRS), and SPAD values of the oilseed rape leaves of 30 plots were measured by a SPAD 502 chlorophyll meter, and the research was carried out at experiment field in the Zhejiang University during growing season from 2007 to 2008. The SPAD 502 chlorophyll meter was applied to investigate the distribution rule of chlorophyll concentration in the oilseed rape. Regression model between the spectral reflectance and chlorophyll concentration (SPAD value) was built by partial least squares (PLS), the correlation coefficient (r), root mean square error of prediction (RMSEP) and bias in prediction set were 0.9368, 3.4992 and 1.834e-07. Correlation between the first derivative of spectral reflectance of oilseed rape leaves and chlorophyll concentration (SPAD value) were analyzed, and the results showed that good correlation coefficient was obtained in the range from 510 to 640 nm and 685 to 720 nm, and the maximum value for correlation coefficient was at the wavelength 707 nm. The linearity equation between the red edge index and chlorophyll concentration (SPAD value) was also analyzed, with the correlation coefficient of 0.986. It is concluded that Vis/NIRS combined SPAD 502 chlorophyll meter was a promising technique to monitor nitrogen status in oilseed rape.

Keywords: Oilseed rape, Visible and near infrared reflectance spectroscopy (Vis/NIRS), Partial least squares (PLS), SPAD value

INTRODUCTION Since the middle of the last century, oilseed rape growing has been increased from 2.7 million hm² to over 30 million hm². The rapeseed oil becomes an important source of the edible oil (Yang et al., 2003). Nitrogen is one of the most critical nutrients for oilseed rape growth. An appropriate nutrient management can improve the oilseed rape yield and quality. In order to implement the site-specific fertilizing of nitrogen, the spatial and temporal variations of the nitrogen of oilseed rape during growing need to be determined.

Several methods are available for assessing the nitrogen status of the crop. Kjeldahl nitrogen determination method is accurate using tissue and chemical analysis, but it is time-consuming and specialized personal is needed. Others are fast measurement techniques including chlorophyll meter (soil plant analysis development, SPAD) and remote sensing techniques. The SPAD meter is a fast and nondestructive diagnostic
tool, which is based on the regression between the light transmittance and foliar N of crop leaf (Chapman & Barreto, 1997). This method has been successfully used to determine nitrogen status in many crops such as corn, wheat, maize, cotton and others (Wu et al., 1998; Daughtry et al., 2000; Arregui et al., 2006). In addition, visible and near infrared spectroscopy was also used to nondestructive detection of nitrogen in Chinese cabbage leaves, and stepwise multiple linear regression (SMLR) showed the highest correlation determine coefficient ($r^2$) of 0.846 (Min et al., 2006). Different spectroscopic techniques have proved the potential of reflectance data for the nitrogen assessing of the crop. The reflectance data acquired from these methods such as hyper-spectral or multi-spectral system is often converted and averaged into reflectance of blue, green, red, near-infrared (450-520, 520-600, 630-690, 760-790 nm) (Nguyen & Lee, 2006). Hence, most of the studies developed regression models based on vegetative indices derived from several spectral bands (Best & Harlan, 1985; Aparicio et al., 2000). Some researchers reported that the light absorption by chlorophyll at the wavelengths of 430, 450, 650 and 660 nm and light reflectance at 550 nm made leaves look green (Min et al., 2006). However, it is believed that the unique information of the unused bands in the case of hyper-spectral or spectroscopic techniques is largely lost in a specific narrow band. The acquired data for one object by spectral scanning always contain several hundreds of variables. Hence, it is very crucial to use multivariate calibration methods to extract the relevant part of the information for the very large data and produce the most reliable models compared to only regression model with several bands. Several common methods including principle component regression (PCR), SMLR, and partial least squares (PLS) regression have been developed for data mining in agricultural applications (Martens & Naes, 1989; Cen et al., 2006). PLS is the most commonly used multivariate calibration method applied in assessing of agricultural product quality and plant nutrition (Ehsani et al., 1999; Fassio & Cozzolino, 2003).

In this paper, the distribution character of oilseed rape was investigated by SPAD 502 chlorophyll meter. The correlation between chlorophyll concentration (SPAD value) and the spectral reflectance of oilseed rape leaves were analyzed using regression model built by partial least squares (PLS). The correlation between the first derivative of spectral reflectance of oilseed rape leaves and chlorophyll concentration (SPAD value) were also analyzed. The visible and near infrared reflectance spectroscopy (Vis/NIRS) method for nitrogen estimation was developed.

MATERIALS AND METHODS

Experimental Design The field experiment was conducted at the experiment farm of Zhejiang University in the 2007/2008 oilseed rape-growing season. The experimental design was the quadratic regression orthogonal design, with one variety of oilseed rape (Brassica Napus), three factors including nitrogen (N), phosphorus (P) and potassium (K), five rates of N, P and K, and two replicates. In this paper, only N was investigated. The five N concentration rates were 0, 90, 135, 180 and 270 kg N/ha, and the standard level was 180 kg N/ha. The number of plots was 30 (including replicates) and the plot size was 1.6×5 m.

Field Data Acquisition The field data was acquired between 10: 00 am and 14: 00 pm to eliminate the variation of the sun azimuth. Five plant leaf samples of each plot were selected and a total of 150 samples were obtained for spectral measurement. All leaf samples were used for Vis/NIRS analysis at 325-1075 nm. For each sample, three reflecting spectra were taken with a field spectroradiometer [FieldSpec® HandHeld (HH), VNIR (325-1075 nm), Analytical Spectral Devices (ASD), Inc., Boulder, CO],
using RS² V4.02 software for Windows designed with a Graphical User Interface (GUI) from ASD. The instrument uses a sensitivity 512-element, photo-diode array spectroradiometer, with the resolution of 3.5 nm. The scan number for each spectrum was set to 10 at the same position, thus a total of 30 individuals were properly stored for later analysis. Considering its 25° field-of-view (FOV), the spectroradiometer was placed at a height of approximately 200 mm and 45° angle away from the surface of the certain oilseed rape leaf. To achieve the relative reflectance measurements, the white reference (a white panel purchased with the spectroradiometer used as white reference) was collected before scanning samples until obtain a nice, clean, 100 % reference line.

A Minolta SPAD-502 chlorophyll meter was used to measure the chlorophyll concentration of the oilseed rape leaf, and SPAD readings for each sample were used as the referenced concentration of nitrogen status.

**Data Pretreatment** Due to the potential system imperfection, obvious scattering noises could be observed at the beginning and end of the spectral data. Thus, the first and last 75 wavelength data were eliminated to improve the measurement accuracy. In addition, the influence of chlorophyll concentration to spectra was primary at the visible region, so the wavelengths from 400 to 800 nm were analyzed in the following. The above spectral data pre-processing was finished in ViewSpec Pro V4.02 (Analytical Spectral Device, Inc.). After that, the spectral data was preprocessed by the Savitzky-Golay smoothing with a window width of 7 (3-1-3) points, and then was the use of the multiplicative scatter correction (MSC) (Helland et al., 1995). The pretreatments were implemented by “The Unscrambler V9.6” (CAMO PROCESS AS, OSLO, Norway).

**Partial least squares (PLS)** PLS is a bilinear modeling method where the original independent information (X-data) is projected onto a small number of LVs to simplify the relationship between X and Y for predicting with the smallest number of LVs.

In the development of PLS model, calibration models were built between the spectral data and the SPAD values, full cross-validation was used to evaluate the quality and to prevent overfitting of calibration models. The optimal number of latent variables (LVs) was determined by the lowest value of predicted residual error sum of squares (PRESS). The prediction performance was evaluated by the correlation coefficient (r), the root mean square error of calibration (RMSEC) or prediction (RMSEP) and bias. The ideal model should have higher r value, lower RMSEC and RMSEP values, and lower bias. The models were carried out by “The Unscrambler V 9.6” (CAMO PROCESS AS, OSLO, Norway).

**RESULTS AND DISCUSSION**

**Chlorophyll Concentration in the Oilseed Rape Plant** The SPAD values were varied by the different growing periods of oilseed rape and the position of the growing leaf. In order to be able to choose a position as a representative, the distribution of chlorophyll concentration in the entire oilseed rape plant was interesting to be known.

SPAD values varied from the different ramus in the same oilseed rape plant, and were different from the top leaf to the middle one in the same ramus, which follow the rule of the top SPAD values was larger than the middle one. In the different ramus, the SPAD values of the bottom ramus were larger than the top one, and it follows the
transportation rule of nitrogen in the oilseed rape plant. The nitrogen in the oilseed rape always transform from the bottom leaf to the top one.

The oilseed rape leaf will grow big gradually and age to death in the growing period. In order to increase the measuring period, the third ramous of the oilseed rape from top was chosen as the tracking object. In the measurement period, the bottom leaves were expanding and belonging to mature leaves, which were aging to death easily, so the bottom leaf was never chosen. The top leaves belong to incomplete spreading leaf, cannot reflect the situation of the whole oilseed rape plant perfectly. So in the measurement period, the third ramous of the oilseed rape from top was chosen as the tracking object on measurement day of March, 21st, 2008.

**Chlorophyll Concentration in the Oilseed Rape Leaf** The distribution of SPAD values in the same leaf was different. The top SPAD values were larger than the bottom ones. The distribution of SPAD values at both sides was asymmetric, with one side approximately larger than the other side. In the same side, the SPAD values became smaller from the top to bottom. During measurement, the SPAD value for the entire leaf was obtained as an average value. Table 1 shows the tracking measurement result for 15 different regions of the third ramous oilseed rape.

The trend of chlorophyll concentration was diminished as time went on. In individual plot the SPAD values measured on March 31st were larger than those measured on March 21st, such as plot 3, 5, 12, 14. After the grouting period of oilseed rape, the chlorophyll concentration of leaf was tend to diminish in all plots from April 6th to the end of the measurement. Generally, the growing periods for oilseed rape can be divided into six phases, including planting, seeding, developing, budding, flowering and grouting. On the first and middle ten days of March, oilseed rape was approximately in the flowering phase. The oilseed rape plant developed persistently, and the ability for photosynthesis was increased ceaselessly. In the grouting phase, the nutrition of leaves started transporting to fruit corner, and the chlorophyll concentration diminished ceaselessly. So during the whole growing period of oilseed rape, the chlorophyll concentration follows the rule of increasing and then diminishing. In our study, the beginning of the measurement was from flowering phase (March 21st), so the chlorophyll concentrations of most plots were already reached maximum or exceeded maximum, the chlorophyll concentrations was at diminishing phase. The SPAD values for March 31st were larger than March 21st in some plots may affect by the factors, like the flowering phase was relatively long, the leaves were still at the developing phase and so on.

**Spectral Reflectivity Property of Oilseed Rape Leaves** In the visible region, the ability for photosynthesis increased from planting phase to flowering phase, because of the ceaselessly growing of oilseed rape plants. After flowering, the nutrition of leaves started transporting to fruit corner, and the chlorophyll concentration diminished gradually. The reflectivity at red and blue ranges started rising. After the fruit corner matured, the lower leaves started shedding continuously, and the nutrition of leaves transported to fruit corner. At the same time, the chlorophyll started decomposing, and the photosynthesis ability for leaves was weakened. The chlorophyll reduced rapidly after supplying nutrition to fruit corner, and the reflectivity at red and blue ranges rose rapidly. At this time, the reflectivity at green waveband was still larger than the reflectivity at red and blue ranges, and there existed a small reflective apex in the visible region. The reflectivity at red and blue wavebands increased gradually as the oilseed rape growing boosted. From the red range to near infrared one, the reflectivity increased gradually as the leaf area index
augmented. When the leaf area index increased to a certain value, the reflectivity went to stable. The internal structure of leaves started changing when fruit corner developed. At the same time, the near infrared reflectivity started declining gradually, until the oilseed rape plant became maturity.

**Data Pretreatment** Generally, the SPAD 502 chlorophyll meter measures the chlorophyll concentration around the verge of oilseed rape leaf and it is not corresponding to the spectral curves collected. Thus, ruinous experiment was done to find the relationship between the chlorophyll concentration for verge and middle of the leaves. SPAD 502 chlorophyll meter was used to measure the verge of eight random choosing leaves from different plots. In each leaf, it measured 30 points and used the averaging values as the final concentration. The verge of oilseed rape leaf was torn and the middle chlorophyll concentration values were obtained, Table 2 shows the fringe and middle SPAD values for eight oilseed rape leaves. After that, the linear regression between the fringe and middle chlorophyll concentration values was analyzed. The result shows the correlation was high. The correlation coefficient was 0.981. The middle SPAD values could be obtained by measuring the SPAD values of verge. So, the SPAD values used later were preprocessed by this formula. The final data used to regression analysis were the average values of the fringe and middle SPAD values.

**PLS Regression Model** PLS model between spectral reflectivity and chlorophyll concentration was developed after the spectral data preprocessed by S.Golay smoothing and MSC. Different LVs were applied to build the calibration models, and no outliers were detected in the calibration set during the development of PLS models. Among 150 samples, 125 samples were used as calibration sets and the left 25 samples as the prediction sets. Among calibration models, the models with 5 LVs turned out to be the best for prediction SPAD value by comparison using aforementioned evaluation standards in section 2.4. In the prediction models, the correlation coefficient ($r_p$), RMSEP and bias by optimal PLS models were 0.9368, 3.4992 and 1.834e-07.

**Correlation Analysis Between the Wavelengths and Chlorophyll Concentration** In the following, the correlation between the wavelengths and SPAD value was analyzed. It would be helpful to examine how SPAD value is simply related to individual wavelength so that a better understanding of Vis/NIRs reflectance may be obtained.

The reflectance data of oilseed rape leaf may be affected by the height of spectroscopy, the variety of illumination intensity, and background factors of different regions. In order to eliminate the influence of background, and clearly reflect the spectral variation properties, the original spectral reflectivity was disposed with first derivative. The reflectivity values after first derivative were averaged by ten from wavelengths 400 to 800 nm, and 40 data were obtained in all. The correlation between chlorophyll concentrations (SPAD values) was built by SPSS12.0 software. The SPAD value changed dramatically over wavelengths from visible spectral region to near infrared spectral region. Generally, the SPAD value showed negative correlation with reflectance mainly at the wavelength region from 430-600 nm and 670-700 nm, and positive correlation mainly from 580-775 nm. Wavelength near 460 nm, 500 nm, 550 nm, 690 nm has higher negative correlations, and near 670 nm, 720-760 nm have higher positive correlations. It included two wavelengths just matched with green peak and red edge, which took an important part in the assessing of nitrogen status.
Wavelength regions showing high correlation indicated that reflectance at these wavelengths might be important for the SPAD value.

**The Relationship Between the Red Edge and Chlorophyll Concentration** The red edge was the wavelength corresponding to the maximum slope of reflected spectral curve, which was caused by intensive absorption of red waveband and intensive reflection of near infrared wavebands. A red edge property was usually described by red edge swing and red edge position. Generally, it lies on wavelengths between 680 to 750 nm, namely the variation from low values range of red wavebands to high values range of reflectivity in near infrared wavebands. Some researchers indicated that there existed relationship between red edge and chlorophyll concentration (Filella & Penuelas, 1994; Smith et al., 2005). The red edge was sensitive to the chlorophyll concentration, and it was the best method to measure the chlorophyll content by spectral techniques. Wang et al. (2001) found a high correlation between chlorophyll content of top leaves for rice (Wang et al., 2001). Others believed this relationship was not notable to foliage canopy, and the correlation between red edge and chlorophyll content was not obvious. Fang et al. (2007) reported the red edge combined green apex was better than red edge to predict the chlorophyll content for oilseed rape leaves (Fang et al., 2007). So, the correlation between the red edge and chlorophyll concentration was analyzed in this study. Table 3 shows the maximum values for the first derivative of the red edge taken from live oilseed rape plant.

Set the chlorophyll concentration values as independent variable X, and red edge inflexion as dependent variable Y, the linear regression equation by linear regression analysis was obtained:

\[ y = 0.7256x + 683.69 \]

The relativity between red edge inflexion and chlorophyll concentration was high, as the correlation coefficient was obtained at 0.986. So it was feasible to analyze chlorophyll concentration of oilseed rape leaves quantitatively using the spectroscopy.

**CONCLUSION** The distribution rule of chlorophyll concentration in the oilseed rape was measured by the SPAD 502 chlorophyll meter, and the third ramous of the oilseed rape from top was measured, and the variation rule for the chlorophyll concentration in different growing periods was analyzed. The spectral reflectivity property of oilseed rape leaves in different growing periods was analyzed, and it indicated that the spectral reflectance for the leaves were smaller in the visible region and bigger in the near infrared region before flowering, but with reverse after flowering. Regression model between the spectral reflectance and chlorophyll concentration was built by PLS, and the \( r_p \), RMSEP and bias in prediction set were 0.9368, 3.4992 and 1.834e-07. The correlation between the first derivative of spectral reflectance of oilseed rape leaves and chlorophyll concentration were analyzed, and the results showed that good correlation coefficient was obtained in the range from 510 to 640 nm and 685 to 720 nm, and the maximum value for correlation coefficient was at the wavelength 707 nm. The linearity equation between the red edge index and chlorophyll concentration was also analyzed, with the correlation coefficient of 0.986. It is concluded that Vis/NIRS combined SPAD 502 chlorophyll meter was a promising technique to monitor nitrogen status in oilseed rape.

**REFERENCES**


APPENDIX

Table 1. The SPAD values for the third ramous of the oilseed rape in different plots and growing period.

<table>
<thead>
<tr>
<th>Date Sample</th>
<th>3.21</th>
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<th>4.06</th>
<th>4.13</th>
<th>4.21</th>
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<td>43.95</td>
<td>44.53</td>
<td>42.13</td>
<td>34.97</td>
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<tr>
<td>2</td>
<td>49.08</td>
<td>48.33</td>
<td>46.27</td>
<td>41.62</td>
<td>36.55</td>
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Table 2. The fringe and middle SPAD values for eight oilseed rape leaves.

<table>
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<tr>
<th></th>
<th>Verge (SPAD values)</th>
<th>Middle (SPAD values)</th>
<th>Verge (SPAD values)</th>
<th>Middle (SPAD values)</th>
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</thead>
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<td>1</td>
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<td>46.0</td>
<td>5</td>
<td>49.8</td>
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<td>2</td>
<td>45.8</td>
<td>47.8</td>
<td>6</td>
<td>48.8</td>
</tr>
<tr>
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<td>44.5</td>
<td>47.8</td>
<td>7</td>
<td>46.1</td>
</tr>
<tr>
<td>4</td>
<td>45.7</td>
<td>47.7</td>
<td>8</td>
<td>50.0</td>
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</table>

Table 3. Position of red edge corresponding to chlorophyll concentration in six regions.

<table>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
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<td>46.5</td>
<td>16.2</td>
<td>9.3</td>
<td>22.9</td>
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<td>red edge position(nm)</td>
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<td>716</td>
<td>715</td>
<td>696</td>
<td>691</td>
<td>698</td>
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