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Chlorophyll Assessment and Sensitive Wavelength Exploration for Tea (*Camellia sinensis*) Based on Reflectance Spectral Characteristics

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Abstract. **A nondestructive method for the determination of chlorophyll index for the tea plant based on reflectance spectral characteristics was investigated. Spectral data were collected from 184 samples with a spectroradiometer in a field experiment. Multivariate analysis techniques, including partial least squares (PLS) and multiple linear regression (MLR), were used for developing calibration models for the determination of chlorophyll index of the tea plant. The best calibration model was achieved using the PLS technique with a correlation coefficient (*r*) of 0.95, a SE of prediction of 3.40, and a bias of 1.9e^{-06}. When the model was used for predicting the unknown samples, good performance was also obtained with *r* of 0.91, SE of calibration of 4.77, and bias of 0.02. Sensitive wavelengths were selected through loading analysis of latent variables in the optimal PLS model, and the validity of these wavelengths was proved by MLR and statistical analysis. Three fingerprint wavelengths (488, 695, and 931 nm) were determined and could potentially be used for developing a simple, low-cost, and efficient instrument for the measurement of chlorophyll index. The results proved the feasibility of reflectance spectra for measurement of chlorophyll index of the tea plant.**

Nitrogen (N) is an important nutrient for tea plant growth to obtain high yield or high quality. An adequate amount of N can accelerate the growth of tea plant, whereas excess N fertilizer may contaminate surface water and groundwater. Accurate assessment of N content of the tea plant is necessary for N fertilization.

Traditional methods for chlorophyll and N determination include soil testing, plant tissue analysis, and long-term field trials. Although these methods are accurate, they are destructive, time-consuming, and expensive. Portable nondestructive meters have been successfully used to determine foliar chlorophyll or N of many plants (Abdelhamid et al., 2003; Castelli et al., 1996; Loh et al., 2002; Schaper and Chacko, 1991; Yamamoto et al., 2002). Chlorophyll meters (e.g., SPAD-502, Minolta, Osaka, Japan) have been used to assess plant N status by measuring transmittance radiation through a leaf at two wavelengths centered near 650 nm and 940 nm (Pinkard et al., 2006). Because the majority of foliar N is contained in chlorophyll molecules, a close relation between foliar chlorophyll content and foliar N content exists (Yoder and Pettigrew-Crosby, 1995). Foliar chlorophyll content is a good indicator of plant N status and photosynthesis activity (Chang and Robison, 2003). Leaf chlorophyll meter readings are generally linear with extractable chlorophyll contents for a wide variety of crops such as sugar maple leaves (van den Berg and Perkins, 2004), four species of hardwoods (Chang and Robison, 2003), and corn (Ulson et al., 2002). Furthermore, Song et al. (2002) studied the relationship between SPAD reading and chlorophyll content of fresh tea leaves and concluded that SPAD reading is correlated with chlorophyll content. Furthermore, high correlation exists between SPAD reading and ratio of chlorophyll a to chlorophyll b.

Although the chlorophyll meter provides a relative N assessment without complex laboratory analysis, 15 or more readings are needed and averaged to get one measurement. Collecting the data of N content for large fields with spatial variations is a time-consuming process using a chlorophyll meter (Daughtry et al., 2000). The measurement of leaf spectral reflectance using a spectrometer is a noncontact and nondestructive approach, and the scan time of one measurement can be less than 1 s; it is a promising method for fast sensing of N status (Bausch and Duke, 1996; Tumbo et al., 2002). Bausch and Duke (1996) used a mobile system to acquire spectral data of corn and found that the N reflectance index had a good correlation (r^2 = 0.81) with chlorophyll meter readings. Tumbo et al. (2002) assembled a spectrometer on a mobile tractor for fast measurement of chlorophyll index and found that spectral reflectance response patterns from a mobile sensor with speed of 0.6 km·h^{-1} could be used to predict chlorophyll in corn, and a strong relationship was obtained between near infrared/green (NIR/G) ratio and chlorophyll meter readings (in SPAD units). Several studies have shown that a direct relationship existed among spectral reflectance, chlorophyll content, and N status in green vegetation. Takebe et al. (1990) obtained a good correlation (r^2 = 0.90) between the chlorophyll meter and spectral reflectance of rice. The spectrophotometer has been used to detect N content of cabbage seedling based on the reflectance spectra of leaves, and a good correlation (r_c^2 = 0.89) was obtained with significant wavelengths (566, 574, 1396, and 1530 nm) (Chen et al., 2004). Karimi et al. (2005) found a high accuracy of discrimination using hyperspectral reflectance spectra for plots with different N treatments. Min and Lee (2005) developed a N sensor for citrus trees based on diffuse reflectance of leaf samples; their calibration models showed a strong relationship between actual N concentration and reflectance spectra of citrus. For another economically important plant, tea, Hu et al. (2006) investigated the relationship between spectral reflectance and chlorophyll meter reading of fresh tea leaf and concluded that it was applicable to detect chlorophyll index based on reflectance spectra. However, the correlation coefficient was relatively low and the measurement SE was large. At the same time, Ishikawa et al. (2006) focused on the determination of chlorophyll of the tea plant by spectral image; they concluded that spectral image made it possible to study differences of growth stage of the tea plant. However, spectral image analysis is more complex compared with the reflectance spectra technique.

In this study, the relationship between visible/near infrared (Vis/NIR) reflectance spectral characteristics of tea plant and chlorophyll index was studied, and more attention was focused on evaluating the performance of calibration models, selecting the proper data pretreatment method, and exploring sensitive wavelengths. The specific objectives were: 1) to evaluate the potential of reflectance spectral characteristics for determining the chlorophyll index of six tea cultivars with a portable spectrophotometer; 2) to explore an effective pretreatment method to enhance the signal-to-noise and reduce noise caused by field environment; and 3) to seek the optimum sensitive wavelengths that are strongly related with the chlorophyll index of the tea plant.

Materials and Methods

Six cultivars of tea plants were selected for this experiment, including *C. sinensis* cv. Jiukeng (JK), *C. sinensis* cv. Biyun (BY),

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C. sinensis cv. Juhuaxiang (JH), *C. sinensis* cv. Lvyafoshou (LY), *C. sinensis* cv. Meizhan (MZ), and *C. sinensis* cv. Zhenghedabaicha (ZH). The samples of the six cultivars were planted in the Seed Resource Garden of Tea Plant in Zhejiang University (long. 120.19° E, lat. 30.26° N), China. These cultivars were planted at two blocks with three cultivars in each block. Within each block, three cultivars were planted in adjacent rows. The physiological age of these plants is ≈30 years, the soil is red loam, and all the tea plants were treated with the same level of N fertilizer (90 kg·ha^{-1} urea). One hundred eighty-four leaves (samples) were randomly selected from ≈60 tea plants and approximately three samples, which were close to the top of the plant were pricked off from each plant. To add the diversity of samples, leaves with different physiological ages were taken into the experiment. The detailed information about the six cultivars is presented in Table 1.

On 20 April and 23 May in 2006, the field experiment was conducted. Reflectance spectra were acquired from 930 to 1630 under clear sky conditions using a field spectroradiometer (Fieldspec HandHeld; Analytical Spectral Devices, Boulder, CO) with 3.5-nm full-width-half-maximum spectral resolution and wavelength range from 325 to 1075 nm. The spectroradiometer was stabilized on a tripod with 45° angle between the spectroradiometer and the horizontal line. The spectroradiometer was set up at ≈100 mm above the surface of the leaf sample with a 10° field of view. Sun was the light source. The zenith and azimuth angle of the sun changed during the experiment period, from 0° to 90° and from –180° to 180°, respectively. To reduce the effect of solar radiation intensity variation, the spectroradiometer was calibrated every half hour by a 100-mm^{-2} optical reference standard panel with ≈100% reflectance across the entire spectrum. Reflectance was computed with measurements from both the leaf sample and a reference standard panel. For each sample, a mean spectrum was averaged by 60 scans. The reflectance spectra of all samples were transformed to absorbance [log (1/R)] values according to Beer-Lambert law (Williams and Norris, 2001). Typical absorbance spectra of samples were shown in Figure 1. The first 75 and the last 75 wavelengths values were cut off as a result of significant noise observed, and all analyses were based on the range of wavelengths between 400 and 1000 nm. After spectra measurement, the relative chlorophyll content was determined by a Minolta SPAD-502 (Minolta, Osaka, Japan) chlorophyll meter. The SPAD sensor was clipped on leaf mesophyll tissue instead of veins. Thirty measurements were taken per sample and averaged to provide a single chlorophyll index.

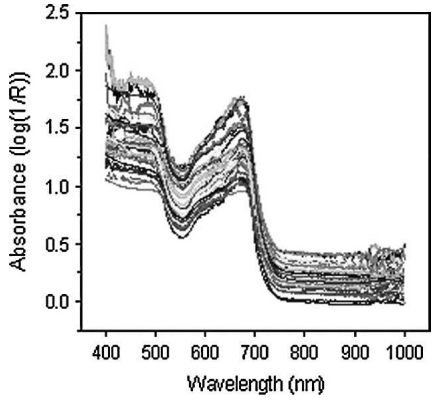

Fig. 1. Typical absorbance spectra of fresh tea leaves.

To test the influences of pretreatment methods on the performance of the calibration models, several types of pretreatments were used, including smoothing, standard normal variate, multiplicative scatter correction, derivatives, and offset (Candolfi et al., 1999). These pretreatments and calculations were carried out using The Unscrambler V9.2 (Camo, Process, AS, Oslo, Norway), a statistical software package for multivariate calibration.

All 184 spectra were divided into training set and prediction set. To avoid bias in subset selection, all samples were sorted according to their respective *Y*-value (chlorophyll index), then one of three samples were selected as the prediction set, resulting in 124 spectra of training set and 60 spectra of prediction set with ≈2:1 division (Table 2). The range of *Y*-value in the training set covered the range of that in the prediction set; therefore, the distribution of the samples was appropriate in the training set and prediction set.

Partial least squares (PLS) is a very powerful and classical multivariate analysis technique in chemometrics (Gomez et al., 2006; Lammertyn et al., 1998; Min and Lee, 2005; Zou et al., 2007). Compared with multiple linear regression (MLR), the advantage of PLS is that it is a bilinear modeling method in which the original *X* (predictor variables) is projected onto a small number of orthogonal latent variables (LVs) to simplify the relationship between *X* and *Y* (response variables) (Lattin et al., 2003) and mitigate the colinearity problem (Helland, 2001; Min and Lee, 2005). In contrast to principal component regression (PCR), the selection of LVs in PLS is more rational. In detail, PCR chooses LVs with the maximum variance in *X* but does not consider the *Y*, but the PLS method balances the two aspects and seeks the LVs that explain both *X* and *Y*. The optimal number of LVs was generally chosen when the minimum of predicted residual error sum of squares (PRESS) value was obtained using full crossvalidation. The PRESS value was the sum of the squared difference between actual and predicted concentrations (during crossvalidation) (Madan et al., 2005). In this study, PLS regression was adopted on the full spectra (400 to 1000 nm) of all 184 samples to explore the relationship between reflectance spectral characteristic and chlorophyll index.

The MLR model is a linear combination of *X* variable that corresponds as closely as possible to the *Y* variable (Lattin et al., 2003). Comparing with PLS and PCR, the drawback of MLR is that the number of samples for MLR must be greater than the number of variables (Madan et al., 2005), and the possible correlation among *X* variables cannot be eliminated. The advantage of MLR is that a direct, simple, and clear relationship between *X* and *Y* can be obtained through MLR analysis. In this study, a small number of sensitive wavelengths from PLS analysis were set as predictor variables (*X*), and the size of these variables was much smaller than that of samples. The MLR model was used to build a direct, simple, and linear formula between *X* and *Y*.

The performances of the PLS and MLR models were evaluated by the SE of calibration (SEC), the SE of prediction (SEP), and the correlation coefficient (*r*) between the actual and predicted concentration. For SEC, a leave-one-sample-out crossvalidation was performed. A good model should have a low SEC, a low SEP, a high *r*, and also a small difference between SEC and SEP. A large difference indicates that too many LVs are used in the model and the noise is included to the model (Gomez et al., 2006; Min and Lee, 2005; Zou et al., 2007). SEC and SEP are calculated using Eqs. [1] and [2], respectively.

$$\mathrm{SEC}\,(\%) = \sqrt{\frac{1}{I_c - n - 1}\sum_{i=1}^{I_c}(\hat{y}_i - y_i)^2} \qquad [1]$$

$$\mathrm{SEP}\,(\%) = \sqrt{\frac{1}{I_p - 1}\sum_{i=1}^{I_p}(\hat{y}_i - y_i - bias)^2} \qquad [2]$$

where $\hat{y}_i$-predicted value of the itch observation, y_i measured the value of the *i*th observation, I_c = number of samples in the calibration set, n = number of independent variables in calibration, I_p = number of

Table 1. Detailed information of the test plants.

Cultivar	Color of the leaf	Duplicate	SPAD $_{mean}$[z]
C. sinensis cv. Jiukeng	Green, lucidus	32	36.7
C. sinensis cv. Biyun	Green	30	31.4
C. sinensis cv. Juhuaxiang	Kelly, lucidus	31	42.9
C. sinensis cv. Lvyafoshou	Pea green	30	41.2
C. sinensis cv. Meizhan	Bottle green,	31	62.2
C. sinensis cv. Zhenghedabaicha	Thick green, lucidus	30	46.9

[z]The mean chlorophyll reading of all samples for each cultivar.

Table 2. Statistical parameters of chlorophyll index (Y).

Sample sets	SN[z]	Minimum	Maximum	Mean	SD
Total samples	184	26.8	75.9	43.494	10.844
Calibration	124	26.8	75.9	43.460	11.142
Prediction	60	29.1	68.6	43.778	10.231

[z]Sample number.

samples in the validation/prediction set, and bias = systematic difference between actual and predicted values calculated by formula

$$\text{bias} = \frac{1}{I_p}\sum_{i=1}^{I_p}(\hat{y}_i - y_i) \qquad [3]$$

Results and Discussion

Figure 1 shows typical absorbance spectra for the fresh tea leaves. All the samples of six cultivars had similar characteristics in absorbance spectral curves. From 400 to 500 nm, the spectral curves were relatively flat and the absorbance values were at the highest level. After 500 nm, spectral curves began to decrease until a "V" shape appeared at 550 nm. From 550 to 675 nm, the absorbance values increased along with the wavelength with a peak at 675 nm. Then the spectral curve decreased again until the minimum was reached at 750 nm. From 750 to 1000 nm, the spectral curves were flat. It can be concluded that the leaf absorbed blue (400 to 500 nm) and red (680 nm) light in the visible range, as reported in previous literature (Datt, 1999) and reflected NIR light (750 to 1000 nm).

In Table 1, there are some differences in leaf color of these six cultivars. For example, the leaves of 'Meizhan' and 'Zhenghedabaicha' are greener than that of other cultivars. It can be found that the degree of greenness is generally consistent with the chlorophyll meter reading for cultivar; in other words, the cultivar with greener leaves usually has a higher chlorophyll reading. To develop a model with high adaptability, all the samples of the six cultivars were considered as a group for building the calibration model without building a model for each cultivar, although the diversity of samples from six cultivars provided some help for developing a model with good stability and high performance.

Considering the different spectral pretreatments, several calibration models were constructed based on the use of PLS. The results of these calibration models are summarized in Table 3. In model 9, r was very low and the values of SEC and SEP were much larger than that of the other models. This result indicated that the correlation between spectral characteristics and chlorophyll index was decreased by the second derivation spectral pretreatment. High-frequency noises in the spectra resulting from the reflectance mode in the field experiment could be magnified through the second derivation pretreatment (Candolfi et al., 1999; Zeaiter et al., 2005). The second derivation pretreatment was not suitable for these spectra.

Model 6 processed by offset correction was evaluated as the best model compared with other models (Table 3). The correlation coefficient of model 6 is the highest, the SEC and SEP values were smallest, and the difference between SEC and SEP values was relative small. The plots of predicted chlorophyll index versus reference chlorophyll index and their statistical summaries are shown in Figure 2. Compared with a similar study by Hu et al. (2006), the same conclusion that reflectance spectra was strongly correlated with chlorophyll index was obtained, but our results were better than that by Hu et al. (2006); their calibration model was obtained with r of 0.7301 and root mean square error of prediction of 10.0781. The worse performance of model by Hu et al. (2006) may be caused by improper spectral pretreatment. The pretreatment method of second derivation used by Hu et al. (2006) had been proved to be an improper method for processing spectral data of tea leaves in this study.

To better understand the relationship between diffuse reflectance spectra and chlorophyll index, the individual wavelengths were analyzed. The optimum calibration model 6 was obtained based on nine LVs of PLS that contributed to the formation of the model. Table 4 shows the variation of each LV (LV1 to LV9). The first nine LVs accounted for the most variation of the whole original data with 97.997%, and the good performance of model 6 proved that these LVs were closely correlated with the chlorophyll index. How the LV was constructed from the absorbance at entire individual wavelengths was demonstrated by the plot of LV loading in Figure 3, and the critical wavelengths for each LV could be detected. These wavelengths with small absolute loading

Table 3. Results of partial least squares models with different data pretreatment methods.

			Calibration			Prediction			
Model	Pretreatment	LV	r	SEC	Bias	r	SEP	Bias	Dif[w]
1	Smoothing(3)[z]	10	0.96	3.29	2.6e–06	0.90	4.88	0.10	1.59
2	Smoothing(5)[z]	10	0.95	3.45	–8.3e–07	0.89	4.95	0.07	1.50
3	Smoothing(7)[z]	4	0.88	5.33	–2.5e–07	0.86	5.73	0.02	0.40
4	Smoothing(9)[z]	4	0.87	5.33	–3.1e–07	0.86	5.70	0.02	0.37
5	SNV[y]	8	0.95	3.41	5.2e–07	0.89	4.98	0.14	1.57
6	Offset correction	9	0.95	3.40	1.9e–06	0.91	4.77	0.02	1.37
7	MSC[x]	2	0.85	5.84	–2.9e–07	0.83	6.15	0.02	0.31
8	First derivatives	4	0.93	4.02	–5.2e–07	0.86	5.68	0.47	1.66
9	Second derivatives	2	0.46	9.86	–1.7e–07	0.26	10.96	0.41	1.10

[z]Smoothing(♦) indicates that the spectrum is smoothed at ♦ data point.
[y]Standard normal variate.
[x]Multiplicative scatter correction.
[w]Difference between SEC and SEP.
LV = latent variable; SEC = SE of calibration; SEP = SE of prediction.

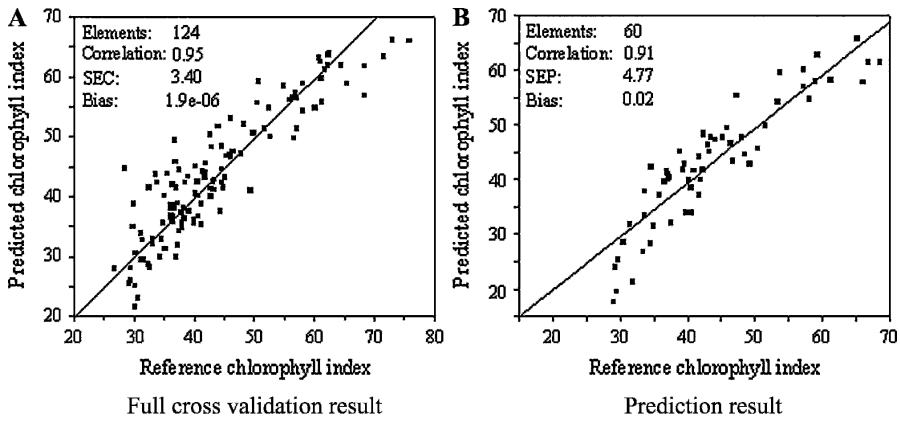

Fig. 2. Results of the optimum calibration model for determination of chlorophyll using partial least squares.

Table 4. Percentage variation explained by the nine latent variables in model 6.

LV[z]	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8	LV9
V[y] (%)	74.25	17.884	3.614	0.278	0.602	0.397	0.306	0.297	0.369
TV[x] (%)	74.25	92.134	95.748	96.026	96.628	97.025	97.331	97.628	97.997

[z]Latent variable.
[y]Variation.
[x]Total variation.

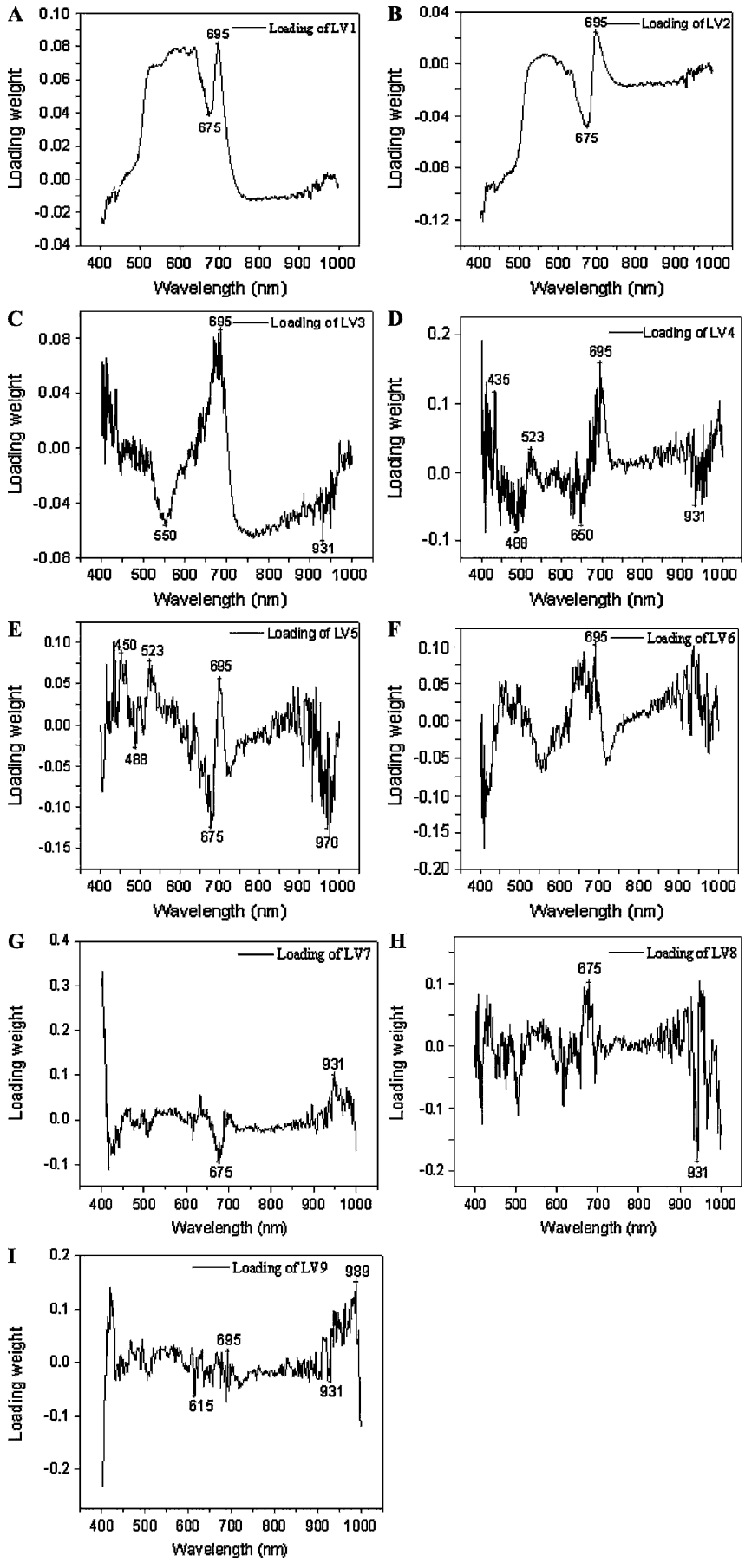

Fig. 3. Loading weights of the first nine latent variables (**A–I**) of the optimum calibration model using partial least squares.

values were less important than those with large values. The loading weight curves had strong peaks and valleys at certain wavelengths, including 435, 450, 488, 523, 550, 615, 650, 675, 695, 931, 970, and 989 nm (Fig. 3A–I). Those individual wavelengths might be of particular importance for determination of the chlorophyll index (Lammertyn et al., 1998). The wavelength 989 nm was not selected as a sensitive wavelength to avoid the noise because it was close to the edge of the wavelength region (1000 nm).

The MLR model was built based on these wavelengths to evaluate their validities [model 10, Eq. (4)]. All 11 wavelengths, including 435, 450, 488, 523, 550, 615, 650, 675, 695, 931, and 970 nm, were set as independent predictor variables and the chlorophyll index was set as the response variable in the MLR model.

$$Y = 17.314 + 21.55 * x_1 - 32.485 * x_2 - 66.813 * x_3 + 95.019 * x_4 - 58.632 * x_5 + 0.456 * x_6 - 12.632 * x_7 + 14.316 * x_8 + 89.355 * x_9 - 60.34 * x_{10} + 0.307 * x_{11} \quad [4]$$

The significance of the MLR model 10 was analyzed by analysis of variance at 99% confidence level (Table 5), and the variables x_3, x_9, and x_{10} were also significant for the model at 99% confidence level. So, a new MLR model was built based on these variables x_3, x_9, and x_{10} [model 11, Eq. (5)].

$$Y = 16.642 - 55.258 * x_3 + 114.428 * x_9 - 64.012 * x_{10} \quad [5]$$

MLR model 11 was statistically significant at 99% confidence level with three significant variables, x_3, x_9, and x_{10} (Table 6). The three corresponding wavelengths, 488, 695, and 931 nm, were considered as the characteristic fingerprint spectra, which were strongly correlated with the chlorophyll index. The wavelength of 695 nm was the most significantly associated with chlorophyll determination. This spectral region had been found to be closely related to chlorophyll content in many plant species such as Chinese cabbage (Min et al., 2006), maple, chestnut, cotoneaster, tobacco (Gitelson and Merzlyak, 1998), eucalyptus (Datt, 1998), and loblolly pine (Carter and Knapp, 2001). The sensitive wavelength 488 nm was very close to the wavelength of 480 nm adopted for the determination of total N by Card et al. (1988). Chlorophyll of fresh tea leaves consists of chlorophyll a and chlorophyll b (Tong, 2000), and chlorophyll a and chlorophyll b mainly absorb light in the range of 400 to 500 nm and 660 to 690 nm, respectively. These two ranges covered the two sensitive wavelengths (488 and 695 nm) found in this study. Among these three sensitive wavelengths, two wavelengths (488 and 695 nm) were in the visible band, and the lights in visible bands had been found

to be effective for determination of chlorophyll by Yoder and Pettigrew-Crosby (1995). The wavelength of 931 nm was not reported as a sensitive wavelength for chlorophyll determination before, and it was contrary with the conclusion by Knipling (1970), in which they reported that reflectance in the NIR region was not correlated to leaf chlorophyll. However, the wavelength (931 nm) might actually be useful for chlorophyll determination of fresh tea leaves, because it was close to the third overtone absorptions band of methylene C-H stretching vibration and methane C-H stretching vibration (Cen and He, 2007).

The correlation coefficient (r) for the MLR model was 0.89 and SEC was 5.13 (Fig. 4A). When the model was used to predict the unknown samples, a reasonable result was obtained with r = 0.86 and SEP = 5.36 (Fig. 4B). The performance of the MLR model was slightly worse than that of the optimal PLS model (Fig. 2). This phenomenon was not consistent with the conclusion by Siesler et al. (2002), in which they reported that MLR model with a few selected spectral variables would frequently outperform the PLS model with full spectral region. This phenomena might be caused by the fact that these diffuse reflectance spectra obtained in this field experiment were interfered with by serious noise and colinearity, and PLS arithmetic can compress data and reduce colinearity between variables (Helland, 2001; Min and Lee, 2005), so the PLS method obtained better performance than the MLR model. However, the MLR model was only based on three individual wavelengths and showed reasonable validity for determination of chlorophyll index, so MLR was also an effective technique for multivariate analysis. In addition, the PLS model provided an approach for wavelength selection that led to a better understanding of diffuse reflectance spectra; the loadings and loading weights of LVs for the PLS model gave the indication of a sensitive wavelength for the predicted Y.

The results showed that it was feasible to determine the chlorophyll index of the tea plant using reflectance spectral characteristics. The multivariable analysis techniques (PLS and MLR) had the potential to develop regression models for assessing chlorophyll index based on spectral data. The proper pretreatment could enhance the quality of the model by filtering the noise. The optimal PLS model was built with r of 0.95, the SEP of 3.40, and a bias of 1.9e^{-06}. The PLS model not only had high prediction accuracy, but also provided an approach for seeking the sensitive wavelengths. The MLR method was effective for selecting the most significant independent spectral variable using analysis of variance and achieved a reasonable result for determination of the chlorophyll index. Three individual sensitive wavelengths (488, 695, and 931 nm) were determined for the measurement of chlorophyll index and could potentially be used for developing a simple, low-cost, and efficient instrument.

Table 5. Analysis of variance table of the MLR model 10 based on all the 11 sensitive wavelengths.

Source of variance	SS[z]	DF[y]	MSS[x]	F-ratio	P value	B-coefficients
Model	1.24E+04	11	1.13E+03	44.197	<0.0001	
Error	2.88E+03	113	25.508			
Adjusted total	1.53E+04	124	123.255			
Variable intercept	362.29	1	362.29	14.203	0.0003	17.374
x_1	55.557	1	55.557	2.178	0.1428	21.55
x_2	69.045	1	69.045	2.707	0.1027	–32.485
x_3	232.23	1	232.23	9.104	0.0032	–66.813
x_4	131.316	1	131.316	5.148	0.0252	95.019
x_5	83.691	1	83.691	3.281	0.0727	–58.632
x_6	5.80E–03	1	5.80E–03	2.27E–04	0.988	0.456
x_7	7.721	1	7.721	0.303	0.5833	–12.632
x_8	26.023	1	26.023	1.02	0.3146	14.316
x_9	632.711	1	632.711	24.804	<0.0001	89.355
x_{10}	428.385	1	428.385	16.794	0.0001	–60.34
x_{11}	1.45E–02	1	1.45E–02	5.67E–04	0.981	0.307

[z]Sum of square.
[y]Degree of freedom.
[x]Mean sum of square.
MLR = multiple linear regression.

Table 6. Analysis of variance table of the MLR model 11.

Source of variance	SS[z]	DF[y]	MSS[x]	F-ratio	P value	B-coefficients
Model	1.22E+04	3	4.07E+03	158.961	<0.0001	
Error	3.07E+03	120	25.586			
Adjusted total	1.53E+04	123	124.16			
Variable intercept	603.809	1	603.809	23.599	<0.0001	16.642
x_3	4.55E+03	1	4.55E+03	177.669	<0.0001	–55.258
x_9	1.20E+04	1	1.20E+04	468.323	<0.0001	114.428
x_{10}	3170	1	3.17E+03	123.97	<0.0001	–64.012

[z]Sum of square.
[y]Degree of freedom.
[x]Mean sum of square.
MLR = multiple linear regression.

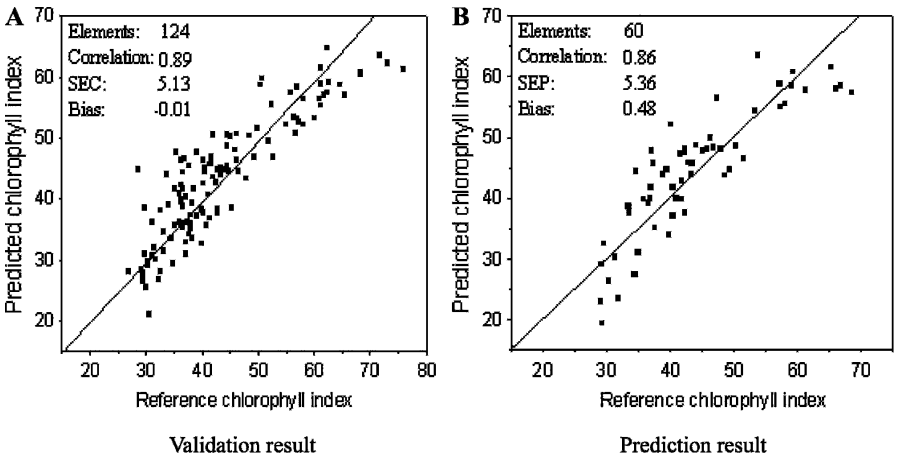

Fig. 4. Results of the calibration model using multiple linear regression and three fingerprint wavelengths.

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