http://www.zju.edu.cn/jzus;　E-mail: jzus@zju.edu.cn
ISSN 1009 - 3095 *Journal of Zhejiang University SCIENCE* V.4,No.6,P.727 - 733,Nov. - Dec.,2003

# *In situ* hyperspectral data analysis for pigment content estimation of rice leaves*

CHENG Qian(程　乾)†, HUANG Jing-feng(黄敬峰),
WANG Xiu-zhen(王秀珍), WANG Ren-chao(王人潮)

(*Institute of Agricultural Remote Sensing & Information Application, Zhejiang University,*
*Hangzhou 310029, China*)

†E-mail: qiancheng525@yahoo.com.cn

Received Dec.2,2002; revision accepted Feb.26,2003

**Abstract:** Analyses of the correlation between hyperspectral reflectance and pigment content including chlorophyll-a, chlorophyll-b and carotenoid of leaves in different sites of rice were reported in this paper. The hyperspectral reflectance of late rice during the whole growing season was measured using a Spectroradiometer with spectral range of 350 - 1050 nm and resolution of 3 nm. The chlorophyll-a, chlorophyll-b and carotenoid contents in rice leaves in rice fields to which different levels of nitrogen were applied were measured. The chlorophyll-a content of upper leaves was well correlated with the spectral variables. However, the correlation between both chlorophyll-b and careteniod and the spectral variables was far from that of chlorophyll-a. The potential of hyperspectral reflectance measurement for estimating chlorophyll-a of upper leaves was evaluated using univariate correlation and multivariate regression analysis methods with different types of predictors. This study showed that the most suitable estimated model of chlorophyll-a of upper leaves was obtained by using some hyperspectral variables such as $SD_r$, $SD_b$ and their integration.

**Key words:** Pigment contents, Hyperspectral reflectance, Rice leaves, Correlation
**Documut code:** A　　**CLC number:** TP7.5; S511

## INTRODUCTION

Pigments are closely related to the physiological function of leaves. Chlorophylls absorb light energy and transfer it into the photosynthetic process. Carotenoids (yellow pigments) can also contribute energy to the photosynthetic system. Because of the importance of pigments for leaf function, the analysis of variations in pigment content may provide valuable information on the physiological state of leaves. Rice yield depends on the physiological function of rice leaves. Most studies showed that the photosynthetic process was accelerated by high chlorophyll content. Therefore, variations of pigment contents are of significant importance for rice growing.

Analysis of leaf reflectance had been carried out extensively to interpret plant foliar chemistry, especially leaf pigments (Thomas and Gausman, 1977; Card *et al*., 1988). Accurate remotely sensed estimates of the foliar biochemical concentration of plants canopies can provide valuable aid to the understanding of ecosystem function over a wide range of scales (Dawson *et al*., 1999). This is because many biochemical processes, such as photosynthesis, respiration, evapotranspiration, and decomposition are related to the foliar concentration of chlorophyll and nitrogen, etc. (Goetz and Prince, 1996).

Recent literature reported that the narrow bands may be crucial for providing additional information with significant improvements over broad bands in quantifying biophysical characteristics of crops. The hyperspectral reflectance data for studies reported in literature were collected by using field spectroradiometers (Blackburn, 1998; Carter, 1998), Compact Airborne Spectrographic Imaging (Gong *et al*., 1995), and NASA-designed Airborne Visible-Infrared Imaging spectrometer (AVIRIS) (Chen *et al*., 1998).

Research contributed by Chappelle *et al*.

---

* Project (No. 40171065) supported by the National Natural Science Foundation of China

(1992) and Penuelas *et al*. (1995) supported a shift towards the application of narrow-band reflectance indices for determining absolute and relative concentration of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), carotenoid (Car) and the total chlorophyll (Chl-tot) in plant leaves.

Evidently, rice is by far the most important genus of Chinese crops. Only few investigations dealt with the relationship between the pigments of rice leaves and the hyperspectral reflectance of rice canopy. Therefore the objectives of this study were to (a) establish relationships between pigment contents of rice leaves and rice canopy hyperspectral reflectance; (b) evaluate the potential of hyperspectral reflectance by establishing vegetation indices, parameters of blue-, yellow- and red-edge, variables indicating reflectance maxima in green and minima in red wavelength as well as by applying area-based predictors for estimating chlorophyll-a contents of upper-leaves; and (c) develop and evaluate models for estimating the chlorophyll-a content of upper leaves.

## MATERIALS AND METHODS

The experiment was conducted in a paddy field located at the Zhejiang University experimental farm, Hangzhou, China (30° 14′ N, 120°10′E) in 1999 and 2000. The mean annual precipitation is 1320.9 mm and the mean annual temperature is 16.2℃. In 1999, rice was sowed on 23 June and transplanted on 24 July. In 2000, rice was sowed on 20 June and transplanted on 10 July. The rice variety was Xiushui 63. The experimental field covered an area of 0.1ha, the plot area was 4 m × 5 m. Five nitrogen fertilizer treatments was 0 kg/ha, 45 kg/ha, 135 kg/ha, 225 kg/ha, 315 kg/ha, and the nitrogen was applied by 60%, 30%, 10% in return to green, elongation and heading stage respectively.

### Spectral reflectance measurement

The canopy spectral reflectance of different nitrogen levels were measured by Analytical Spectral Devices (Fieldspec® ) UV/VNIR (350 nm – 1050 nm) Spectroradiometer in different stages. The measured spectral region ranged from 350 nm to 1050 nm, at resolution of 3 nm. The rice canopy spectral measurements were carried out in tillering, elongation, booting, heading and maturity stages during clear and windless days; and always carried out between 10:00 and 11:45 (Beijing time). The 25° field of view of the sensor was toward the nadir, 0.75m to the rice canopy. The 10 spectral records were averaged to yield a spectral reflectance for each sample. The absolute reflectance factor was obtained by using a white Spectralon panel with spectral reflectance measurements of the rice canopy beforehand and afterwards.

### Pigment contents assay

After spectral measurements of the rice canopy, three fully expanded leaves were immediately extracted from the main stem of rice from top to bottom and the pigment contents were measured. From top to bottom, the first rice leaf was labeled upper leaf, the second leaf middle leaf and the third leaf lower leaf. The contents of chlorophyll-a, chlorophyll-b and carotenoid were analyzed with Bai and Tang (1993).

### Statistical analyses

Multiple statistical analyses and the analyses of hyperspectral variables based on spectral waveband position were mainly used in the hyperspectral reflectance analysis. The hyperspectral variables included the spectral reflectance, the first derivative of reflectance and spectral position based, area based, and vegetation index based variables (Pu and Gong, 2000). The estimation models were constructed using the linear and nonlinear regressions and the stepwise regression analyses.

## RESULTS AND DISCUSSION

### Relationships between pigment contents of rice leaves at different sites of rice and hyperspectral reflectance

The correlations between pigment contents of leaves at different sites of rice and spectral reflectance are presented as correlograms (Figs. 1 – 3). The correlograms were, in general terms, similar to those reported by others (Card *et al*., 1988; Yoder and Pettigrew-Crosby, 1995). The wavelengths of maximum sensitivity to pigment content were indicated by high negative correlation coefficients as reflectance decreased with increasing pigment content at these wavelengths.

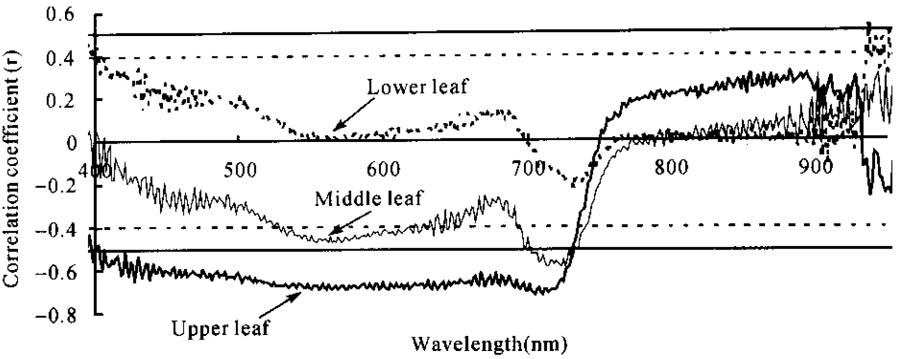

**Fig.1** Correlogram showing the coefficients of the correlation between the spectral reflectance of rice canopy and chlorophyll-a content of leaves at different sites; 95 percent of confidence limits are at ± 0.40 level; 99 percent of confidence limits are at ± 0.51 level

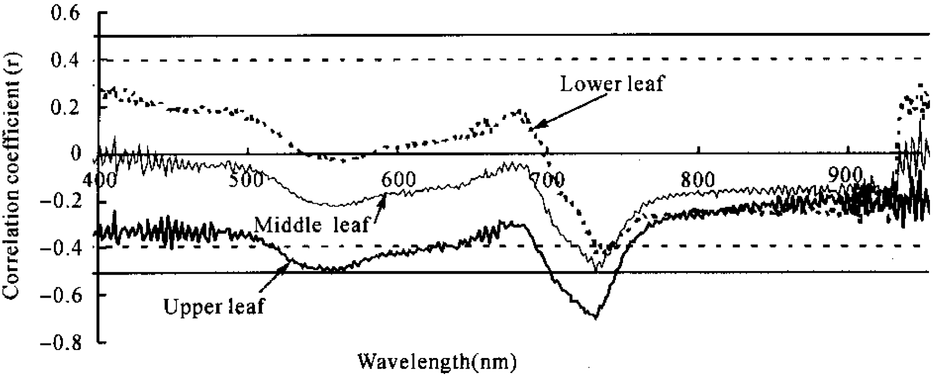

**Fig.2** Correlogram showing the coefficients of the correlation between the spectral reflectance of rice canopy and chlorophyll-b content of leaves at different sites; 95 percent of confidence limits are at ± 0.40 level; 99 percent of confidence limits are at ± 0.51 level

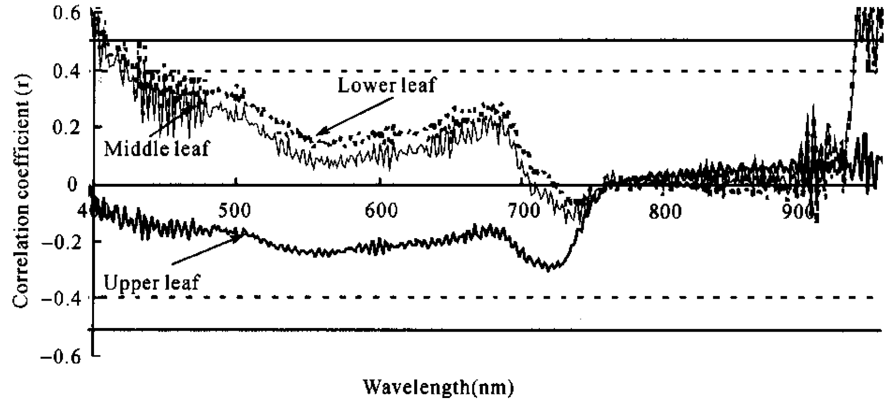

**Fig.3** Correlogram showing the coefficients of the correlation between the spectral reflectance of rice canopy and carotenoid content of leaves at different sites; 95 percent of confidence limits are at ± 0.40 level; 99 percent of confidence limits are at ± 0.51 level

The correlation coefficient of Chl-a and spectral reflectance of the rice canopy decreased from upper to lower leaves (Fig.1). The highest correlation ($r = -0.7$) was calculated for Chl-a of the upper leaves and the spectral reflectance, near 710 nm. The second highest correlation was calculated for Chl-a of the middle leaf and the spectral reflectance. The spectral reflectance was insensitive to the Chl-a of lower leaves at all the relevant wavelengths. Fig.1 shows that the coefficient of the correlation between Chl-a and spectral reflectance was significant at the 0.01 significance level in the visible region.

The correlograms of spectral reflectance versus Chl-b were almost identical to those for Chl-a with maxima occurring at the same wavelength，（$\lambda = 732$ nm），and with the same correlation coefficient of Chl-b of upper leaves and spectral reflectance. The coefficient of the correlation between Chl-b and spectral reflectance decreased from upper to lower leaves（Fig.2）. In the visible region of wavelengths the coefficient of the correlation between Car content and the spectral reflectance was not significant at 0.05 level（Fig.3）.

### Relationships between pigment contents of rice leaves and hyperspectral variables

The content of Chl-tot of upper leaves was strongly correlated with the green peak，red edge and red well，as well as with the vegetation indices calculated from the values of the blue edge and red edge（Table 1）. The coefficient of the correlation between Car content of upper leaves and hyperspectral variables did not exceed the significance level，as well as the coefficient of the correlation between Chl-tot content of upper leaves and $D_b$，$\lambda_b$，$D_y$，$\lambda_y$，$D_r$，$\lambda_g$，$\lambda_o$ and $SD_y$. This indicated that the variation of Chl-tot content of upper leaves did not cause the variation of hyperspectral variables，as mentioned previously.

**Table 1 Correlation between chlorophyll contents of upper leaves and hyperspectral variables（$n = 25$）**

| Variables | Chl-a | Chl-b | Car | Chl-tot |
|---|---|---|---|---|
| $D_b$ | $-0.374$ | $-0.403^{*}$ | $-0.148$ | $-0.424$ |
| $\lambda_b$ | $0.212$ | $0.032$ | $-0.021$ | $0.167$ |
| $D_y$ | $0.270$ | $0.340$ | $0.120$ | $0.324$ |
| $\lambda_y$ | $0.171$ | $0.130$ | $-0.036$ | $0.173$ |
| $D_r$ | $0.072$ | $-0.219$ | $0.006$ | $-0.029$ |
| $\lambda_r$ | $0.601^{**}$ | $0.162$ | $0.190$ | $0.500^{**}$ |
| $R_g$ | $-0.676^{**}$ | $-0.493^{*}$ | $-0.227$ | $-0.678^{**}$ |
| $\lambda_g$ | $-0.144$ | $-0.013$ | $-0.308$ | $-0.111$ |
| $R_r$ | $-0.641^{**}$ | $-0.266$ | $-0.182$ | $-0.567^{**}$ |
| $\lambda_o$ | $0.023$ | $0.195$ | $-0.294$ | $0.089$ |
| $SD_b$ | $-0.606^{**}$ | $-0.578^{**}$ | $-0.256$ | $-0.658^{**}$ |
| $SD_y$ | $0.352$ | $0.453^{*}$ | $0.145$ | $0.426^{**}$ |
| $SD_r$ | $0.262$ | $-0.210$ | $0.033$ | $0.113$ |
| $R_g/R_r$ | $0.373$ | $-0.022$ | $0.161$ | $0.264$ |
| $(R_g - R_r)/(R_g + R_r)$ | $0.397$ | $-0.077$ | $0.131$ | $0.261$ |
| $SD_r/SD_b$ | $0.805^{**}$ | $0.585^{**}$ | $0.331$ | $0.806^{**}$ |
| $SD_r/SD_y$ | $-0.658^{**}$ | $-0.555^{**}$ | $-0.227$ | $-0.687^{**}$ |
| $(SD_r - SD_b)/(SD_r + SD_b)$ | $0.741^{**}$ | $0.446^{*}$ | $0.253$ | $0.708^{**}$ |
| $(SD_r - SD_y)/(SD_r + SD_y)$ | $-0.500^{*}$ | $-0.431^{*}$ | $-0.163$ | $-0.526^{**}$ |

$^{*}$，$^{**}$ refer to the significance at levels 0.05 and 0.01 respectively

The coefficient of the correlation between the chlorophyll content of middle leaves and the wavelength of the green peak，the red edge wavelength and $SD_r/SD_b$ exceeded the 0.01 significance level. The carotenoid content of middle leaves was strongly correlated only with the $\lambda_g$，$\lambda_o$，but not with other hyperspectral variables. Likewise，the coefficient of the correlation between the contents of Chl-a，Chl-b and Car of lower leaves exceeded the specific significance levels only for $\lambda_o$，but not for other hyperspectral variables.

The correlation between chlorophyll contents of leaves and spectral variables evidently decreased from upper to lower leaves. The estimation models of chlorophyll contents of upper leaves can therefore be constructed using the vegetation indices formed by the green peak reflectance，the red well reflectance，the red edge wavelength，the blue edge area and "three edge" area.

## Estimation models of chlorophyll-a of upper leaves by means of hyperspectral reflectance

Estimation model of Chl-a of upper leaves was constructed using the single variable whose correlation coefficients of linear and non-linear regressions were maximal（Table 1）. Table 2 shows that for the variables $R_g$ and $SD_b$，the optimum model is logarithmic，for the variable $R_r$，the optimum model is linear，and for variables $\lambda_r$，$SD_r/SD_b$ and $(SD_r - SD_b)/(SD_r + SD_b)$，the optimum model is exponential.

**Table 2 Linear and non-linear regression between chlorophyll-a contents of upper leaves and the variables of the hyperspectral reflectance（$n = 25$）**

| $X$ | Model | $R^2$ | $F$ |
|---|---|---|---|
| $\lambda_r$ | $Y = -16.916 + 0.0258x$ | 0.363 | 13.04 |
| | $Y = -120.72 + 18.6059\ln x$ | 0.359 | 12.90 |
| | $Y = 8.7 - 0.5\exp(0.0137x)$ | 0.384 | 14.36 |
| $R_g$ | $Y = 2.5318 - 11.507x$ | 0.457 | 19.38 |
| | $Y = -0.2336 - 0.7219\ln x$ | 0.534 | 26.35 |
| | $Y = 3.7091 - 53.094x + 329.268x^2$ | 0.606 | 16.90 |
| | $Y = 4.0516 - 1.264x + 629.717x^2 - 1548.4x^3$ | 0.607 | 10.82 |
| | $Y = 2.6081\exp(-5.9851x)$ | 0.468 | 20.22 |
| $R_r$ | $Y = 2.3359 - 21.803x$ | 0.543 | 27.36 |
| | $Y = -0.0301 - 0.4801\ln x$ | 0.411 | 16.04 |
| | $Y = 2.7402 - 62.566x + 844.121x^2$ | 0.465 | 20.01 |
| | $Y = 3.1556 - 126.52x + 3739.77x^2 - 38912x^3$ | 0.470 | 9.77 |
| | $Y = 2.3597\exp(-11.434x)$ | 0.481 | 6.49 |
| $SD_b$ | $Y = 2.3695 - 0.0559x$ | 0.367 | 13.35 |
| | $Y = 3.1678 - 0.6174\ln x$ | 0.472 | 20.57 |
| | $Y = 3.2013 - 0.2329x + 0.0078x^2$ | 0.546 | 13.24 |
| | $Y = 3.9466 - 0.4710x + 0.0306x^2 - 0.0006x^3$ | 0.559 | 8.86 |
| | $Y = 2.400\exp(-0.0292x)$ | 0.380 | 14.08 |
| $SD_r/SD_b$ | $Y = 1.1873 + 0.0791x$ | 0.647 | 42.25 |
| | $Y = 0.4927 + 0.6588\ln x$ | 0.610 | 35.91 |
| | $Y = 1.2502 + 0.0643x + 0.0008x^2$ | 0.648 | 20.27 |
| | $Y = 2.0333 - 0.2204x + 0.0322x^2 - 0.0011x^3$ | 0.662 | 13.69 |
| | $Y = 1.2995\exp(0.0408x)$ | 0.653 | 43.31 |
| $(SD_r - SD_b)/(SD_r + SD_b)$ | $Y = -0.5074 + 3.0926x$ | 0.549 | 28.04 |
| | $Y = 2.4737 + 2.2440\ln x$ | 0.522 | 25.10 |
| | $Y = 8.9103 - 22.254x + 16.8634x^2$ | 0.655 | 20.89 |
| | $Y = 0.5341\exp(1.6149x)$ | 0.566 | 30.05 |

Stepwise regression analysis was used to explore the relationships between the first derivative of reflectance and the content of Chl-a. Only two wavelengths were selected for detecting Chl-a of upper leaves. The first selected wavelength, with maximum correlation coefficient was 743.37 nm, the second one was 713.66 nm. Likewise, two wavelengths were selected for describing the Chl-a content of middle leaves. The first one, with the maximum correlation coefficient, was 696.68 nm, the second one was 352.86 nm. It was evident that the selected wavelengths for representing Chl-a content of middle leaves can less explain the square error of percentage of Chl-a content than the specific wavelengths of upper leaves. This was the same for the selected wavelengths for describing Chl-a content of lower leaves compared to middle and upper leaves (Table 3).

**Table 3 Stepwise regression between Chlorophyll-a of upper and middle leaves and the variables of the first derivative of reflectance ( $n = 25$ )**

| Step number | Model | $R^2$ | $F$ |
|---|---|---|---|
| 1 | $Y_{\text{upper leaf}} = 1.114 + 0.828\ dR_{743.37}$ | 0.522 | 25.124 |
| 2 | $Y_{\text{upper leaf}} = 2.048 + 0.992\ dR_{743.37} - 1.122\ dR_{713.66}$ | 0.705 | 26.297 |
| 1 | $Y_{\text{middle leaf}} = 2.835 - 1.911\ dR_{696.68}$ | 0.496 | 22.662 |
| 2 | $Y_{\text{middle leaf}} = 3.032 - 2.482\ dR_{696.68} - 0.792\ dR_{352.86}$ | 0.612 | 17.341 |

Derivatives remove the noise effects of the background. The experiments showed that Chl-a had its strongest correlation with $dR_{743.37}$ or $dR_{696.68}$. Therefore 743.37 nm and 696.68 nm can be selected as the characteristic wavelengths for detecting Chl-a contents of rice leaves, and showing strongest correlation with Chl-a and at the same time little noise effects from the background.

## Accuracy analysis of the estimation models of chlorophyll-a content of upper leaves

It was essential to analyze the accuracy of the models of pigment contents, which have been estimated. The standard of the evaluation was as follows:

$R^2$, the square of the correlation coefficient $R$, can be used to evaluate the correlation between spectral reflectance and pigment contents.

$$RMSE = \sqrt{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / n} \qquad (1)$$

Where $y_i$ and $\hat{y}_i$ are measured and predicted values respectively, $n$ is number of samples including predicted and tested ones.

$$RELATIVE\ ERROR\ \% = (y_i - \hat{y}_i) / y_i \times 100 \qquad (2)$$

Where $y_i$ and $\hat{y}_i$ are measured and predicted values respectively.

The model validation was performed using the experiment data from the year 2000 as testing data (Table 4).

**Table 4 Predicted $R^2$ of estimation models of chlorophyll-a content of upper leaves using hyperspectral variables ( $n = 15$ )**

| Type | Estimation models of Chl-a of upper leaves by hyperspectral reflectance | Predicted( $R^2$ ) |
|---|---|---|
| Linear | $Y = -0.2336 - 0.7219\ln R_g$ | $0.3853^{*}$ |
| or | $Y = 2.3359 - 21.8031 R_r$ | $0.5811^{**}$ |
| Non-linear | $Y = 3.2013 - 0.2329 SD_b + 0.0078 SD_b \times SD_b$ | $0.4263^{**}$ |
| Regression | $Y = 1.2995\exp(0.0408 SD_r / SD_b)$ | $0.6537^{**}$ |
| | $Y = 0.5341\exp(1.6149(SD_r - SD_b)/(SD_r + SD_b))$ | $0.6903^{**}$ |
| Step-wise | $Y = 1.1136 + 0.8284 dR_{743.37}$ | $0.4339^{**}$ |
| Regression | $Y = 2.0481 + 0.992 dR_{743.37} - 1.122 dR_{713.66}$ | $0.6556^{**}$ |

$^{*}$, $^{**}$ refer to the significance at levels 0.05 and 0.01 respectively

Predicted $R^2$ value of Chl-a content of upper leaves exceeded the 0.01 significance level, except exponential models with the variable $R_g$. The optimum models of Chl-a content of upper leaves were constructed according to the maximal value of predicted $R^2$.

$$Y = 2.048 + 0.992\ dR_{743.37} - 1.122\ dR_{713.66}$$

The predicted $R^2$ is 0.6556.

## CONCLUSIONS

Analysis of chlorophyll-a, chlorophyll-b and carotenoid contents with hyperspectral variables indicated that red edge, green peak reflectance wavelengths were strongly correlated with chlorophyll and carotenoid contents. The coefficient of the correlation between $SD_b$, $SD_r/SD_b$, $(SD_r - SD_b)/(SD_r + SD_b)$ and chlorophyll-a content of upper leaves exceeded the 0.01 significance level. Relationships between the first derivative of reflectance and chlorophyll and carotenoid content of rice leaves were explored using stepwise regression. Wavelengths with maximum correlation coefficient were selected to describe the contents value for chlorophyll-a or chlorophyll-b related to the red edge.

In this works suitable models for estimation of pigment contents of rice leaves were constructed. The chlorophyll-a content of upper leaves was significantly correlated with the spectral variables. Furthermore, the correlation of chlorophyll-b and carotenoid with the spectral variables was much lower than the correlation of chlorophyll-a with the spectral variables. Hyperspectral remote sensing was showed to be feasible for estimating chlorophyll-a contents of upper leaves of rice. Hyperspectral remote sensing will provide the vital parameters for growth simulation models of rice and for supporting precision farming.

## References

Bai, B.Z. and Tang, X.J., 1993. Testing Technology of Plant Physiology. China Science and Technology Press, Beijing, China (in Chinese).

Blackburn, G.A., 1998. Spectral indices for estimating photosynthectic pigment concentrations: A test using senescent tree leaves. *International Journal of Remote Sensing*, **19**(4): 657 – 675.

Card, D.H., Peterson, D.L. and Matson, P.A., 1988. Prediction of leaf chemistry by the use of visible and near infrared reflectance spectroscopy. *Remote Sensing of Environment*, **26**(2): 123 – 147.

Carter, G.A., 1998. Reflectance bands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. *Remote Sensing of Environment*, **63**(1): 61 – 72.

Chappelle, E.W., Kim, M.S. and McMurtrey, J.E., 1992. Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophylla, chlorophyllb and the carotenoid in soybean leaves. *Remote Sensing of Environment*, **39**(3): 239 – 247.

Chen, Z., Elvidge, C.D. and Groeneveld, D.P., 1998. Monitoring seasonal dynamics of arid land vegetation using AVIRIS data. *Remote Sensing of Environment*, **65** (3): 255 – 266.

Dawson, T.P., Curran, P.J., North, P.R.J. and Plummer, S.E., 1999. The propagation of foliar biochemical absoption features in forest canopy reflectance: a theoretical analysis. *Remote Sensing of Environment*, **67**(2): 147 – 159.

Goetz, S.J. and Prince, S.D, 1996. Remote sensing of net primary production in boreal forest sands. *Agricultural and Forest Meteorology*, **78**(3 – 4): 149 – 179.

Gong, P., Pu, R. and Miller, J.R., 1995. Coniferous forest leaf area index estimation along the Oregon transact using compact airborne spectrographic image data. *Photogrammetric Engineering and Remote Sensing*, **61** (9): 1107 – 1117.

Penuelas, J., Baret, F. and Filella, I., 1995. Semi-empirical indices to assess carotenoids/chlorophylla ratio from leaf spectral reflectance. *Photosynthetica*, **31**(1): 221 – 230.

Pu, R. and Gong, P., 2000. Hyperspectral Remote Sensing and its Applications. Higher Education Press, Beijing, China, p.205 – 206 (in Chinese).

Thomas, J.R. and Gausman, H.W., 1977. Leaf reflectance versus leaf chlorophyll and carotenoid concentration for eight crops. *Agronomy journal*, **69**(2): 799 – 802.

Yoder, B.J. and Pettigrew-Crosby, R.E., 1995. Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400 – 2500nm) at leaf and canopy scales. *Remote Sensing of Environment*, **53**(3): 199 – 211.