

Estimation of Leaf Nitrogen and Grain Protein Content by Hyperspectral Vegetation Index in Winter Wheat

Zhou Wang^{1,2}, Wenjiang Huang^{1,*}, Keming Yang²,
Long Tian⁴, Li Cui², Gunjun Yang³, and Heli Li³

¹The Key Laboratory of Digital Earth Sciences, Institute of Remote Sensing and Digital Earth,
Chinese Academy of Sciences, Beijing 100094, China

²China University of Mining and Technology, Beijing 100083, China

³National Engineering Research Center for Information Technology in Agriculture, Beijing 100097, China

⁴Shanghai Hangyao Information Technology Co. Ltd., Shanghai 200083, China

(Received: 20 March 2012. Accepted: 21 October 2012)

To understand the relationship between hyperspectral vegetation index and wheat leaf nitrogen content as well as grain quality, we studied the correlations between leaf nitrogen content and spectral vegetation indices at different growth stages for two wheat cultivars: high protein content cultivar Zhongyou 9507 and moderate grain protein content cultivar Jingdong 8. At filling stage, significant correlations between wheat leaf nitrogen content and grain protein, dry gluten content were established. Wheat leaf nitrogen content at the different growth stages could be estimated by selected hyperspectral vegetation indices, and the leaf nitrogen content could be further utilized to predict grain protein quality. This research provided some basic understanding for regression models of vegetation index for grain protein prediction by leaf nitrogen content.

Keywords: Wheat, Hyperspectral Vegetation Index, Leaf Nitrogen Content, Grain Protein Content.

1. INTRODUCTION

Wheat is one of the main crops and the second grain yield production in the world. Its quality and quantity mainly depends on nitrogen. At different growth stages of wheat, it has a guiding significance to improving the production and quality that managing the wheat plant, especially the status of leaf nitrogen.¹ Remote sensing is in the forefront of the remote sensing technology.² Yande et al. determined albumen pH of eggs based on visible-near infrared (visible-NIR) diffuse reflectance spectroscopy in the wavelength range of 500–950 nm, and the results show that the visible-NIR spectroscopy in combination with variable selection was a feasible method to detect albumen pH of egg quality non-destructively.³ And in the field of agriculture, getting winter wheat canopy nitrogen information by remote sensing, and making it as the basis of fertilization decision-making and quality evaluation, it has the great potential of economic and ecological benefit.⁴

In recent years, the remote sensing technology had been conducted for the area of crop nitrogen nutrition by many researchers. Li et al. analyzed the correlation between leaf spectra and their nitrogen contents, and established the prediction models by spectral index for nitrogen content in rice.⁵ Tang et al. compared five different kinds of wheat under nitrogen application levels from 0, 100, 200 and 400 kg · N · ha⁻¹, and revealed the relationships among the different kinds of hyperspectral reflection and nitrogen density, LAI, and leaf dry weight. The prediction model of nitrogen abundance at anaphase of winter wheat was established.⁶ Chen et al. introduced the agricultural parameter, Nitrogen Nutrition Index (NNI) in their study. It can be used as an intermediate variable between remote sensing data with grain protein content to improving the accuracy of wheat grain protein content inversion.⁷ Aimed at measuring the change of canopy spectral reflectance, LAI and CCD of rice and wheat in different growth stages, Yang et al. analyzed the correlation between hyperspectral vegetation indices, LAI and CCD, and confirmed the optimum vegetation indices for estimating LAI and CCD

*Corresponding author; E-mail: yellowstar0618@163.com

of rice and wheat.⁸ These studies provided a good understanding about the relationship between spectral reflectance with crop nitrogen level. However, studies on the correlation between wheat and spectral vegetation indices in different growth stages were reported rarely. These studies used quantitative remote sensing to monitor the relationships between vegetation indices and leaf nitrogen content at different wheat growth stages. The information from these studies will provide indirect basis for predicting the grain quality by remote sensing.

2. MATERIALS AND METHODS

2.1. Design of Experiment

The field experiments were carried out in Experiment Base for Precision Agriculture in Xiaotangshan of Changping district of Beijing, China. The soil of the test block is wet. The nutrient content stored in soil from 0 to 20 cm downward is organic matter 1.45%, total nitrogen 0.091%, alkali solution nitrogen 63.4 mg/kg, effective phosphorus 37.7 mg/kg, rapidly-available potassium 123.4 mg/kg. Two local wheat cultivars: high grain protein “Zhongyou 9507” and moderate grain protein “Jingdong 8”. Nitrogen fertilizer as urea was applied at four rates (0 (N0), 150 (N1), 300 (N2), and 450 (N3) kg · N · ha⁻¹) before planting, 50% nitrogen of all treatment were converted into urea and diammonium phosphate and regarded as base fertilizer. Other 50% was applied twice at reviving stage and jointing stage.

2.2. Determination Methods

Leaf nitrogen content was measured by Kjeldahl method using a B-339 type kjeldahl apparatus.⁹ Spectral reflectance was measured by FieldSpec spectrometer developed by U.S. ASD. The field of view was 25°. The spectral reflectance was revised by standard reference board. The wavelength ranges from 350 to 2500 nm. Sampling interval was 1.4 nm that from 350 nm to 1000 nm range, and sampling interval was 2 nm in 1000 to 2500 nm. The weather was sunny and calm. We determined the reflectance vertically from the top of the canopy 0.5 m high at Beijing time 10:30–14:00. Every sample spot was measured for 20 times, and the average was used as the spectral reflectance value of the sample spot. Before and after the determination of each treatment, we revised the reflectance based on the following equation.¹⁰ The solar radiation spectrum of reference board was measured strictly by the transform. We can DN_R^n calculate the $R_T^n = DN_T^n / DN_R^n \times R_R^n$ solar radiation spectrum.

Where R_R^n and R_T^n are spectral reflectance of target and board in the band n . DN_R^n and DN_T^n are sunlight intensity of target and reference board.

2.3. Data Analysis

The correlation between leaf nitrogen content and spectral vegetation indices were analyzed by using the SPSS

software. The coefficient of determination (R²) was used as metrics for explaining the correlation. The root mean square error (RMSE) and prediction relative error (PRE) were used to measure the accuracy of the model.

3. RESULTS AND DISCUSSION

3.1. Dynamic Change Analysis of Leaf Nitrogen Content at Different Growth Stages

As shown in Figure 1, wheat leaf nitrogen content was higher at jointing stage. The leaf nitrogen content of Zhongyou 9507 and jingdong 8 were up to the maximum at elongation (starting) stage, and then decreased after jointing stage until reproductive growth stage. This may be due to relatively high chlorophyll synthesis at early stage, resulting in relatively high nitrogen content.

Compared Zhongyou 9507 with Jingdong 8, the leaf nitrogen content of Zhongyou 9507 apparently decreases more than Jingdong 8. The difference of leaf nitrogen content between the starting stage and milk ripe stage of Zhongyou 9507 was larger than that of jingdong 8. The high seed protein content of Zhongyou 9507 might require a larger amount of nitrogen in leaves so that it can be transferred to the grain for protein synthesis. The significant decrease of nitrogen content hinted the translocation of *N* into protein in grain. The trend of decline of jingdong 8 was not evident before blossom stage, but became significant after blossom stage at starting stage and milk stage, leaf nitrogen content of Zhongyou 9507 had no significant difference under different treatments, but the leaf nitrogen

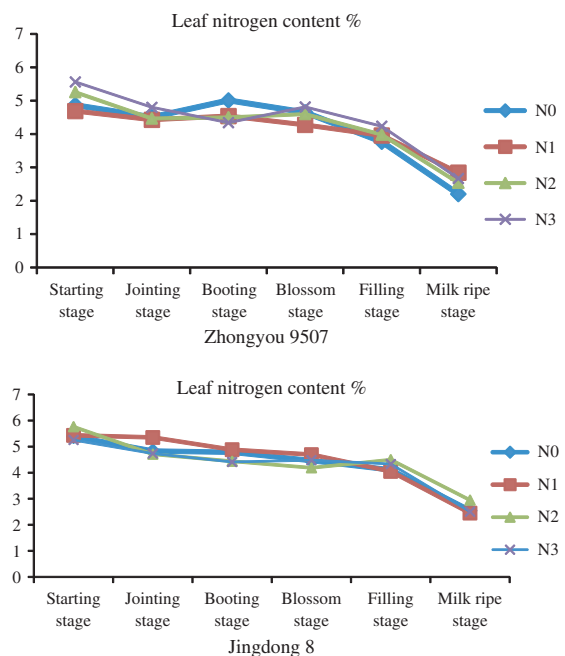


Fig. 1. Dynamic change of leaf nitrogen content at different growth stages.

contents of jingdong 8 has a significant difference, suggesting that jingdong 8 is more sensitive to nitrogen than Zhongyou 9507.

3.2. Correlation Between Leaf Nitrogen Content and Spectral Vegetation Index at Different Growth Stages

The correlation between wheat nitrogen content and vegetation index is very important for forecasting nutritional status. On the basis of previous researches, 11 spectral vegetation indices were selected as shown in Table I.¹¹

The two varieties of Zhongyou 9507 and Jingdong 8 were planted under different soil fertilizer treatments. The leaf nitrogen content was regressed with spectral vegetation index at different growth stages. The regression equations provided as follows.

The correlations between leaf nitrogen content and any spectral vegetation index were not statistically significant at jointing stage, booting stage, blossom stage and grain filling stage for Zhongyou 9507 (Table II). Significant negative correlation was observed between leaf nitrogen content and vegetation index TCARI at starting stage. At milk ripe stage, the relationship was significant between the most of spectral vegetation indices and leaf nitrogen content.

The correlations between leaf nitrogen content of Jingdong 8 and the vast majority of spectral vegetation indices reached significant or extremely significant level (Table II). Significant negative correlation was founded between leaf nitrogen content and vegetation index PPR at jointing and grain filling stage. Blossom stage reached extremely significant level. At booting stage, all the vegetation index correlations did not reach the significant or extremely significant level. At milk ripe stage, the vast majority of spectral vegetation indices were positively correlated to leaf nitrogen content at significant or extremely significant level.

Correlation coefficients between leaf nitrogen content of moderate grain protein variety Jingdong 8 and vegetation

indices were higher than those of high grain protein variety Zhongyou 9507 at elongation stage. However, both varieties are not significance with spectral vegetation indices at booting stage. At jointing stage, blossom stage and filling stage, vegetation index PPR could independently predict wheat leaf nitrogen content for Jingdong 8. While the prediction showed inconsistency for Zhongyou 9507. Correlations between leaf nitrogen content and the most spectral vegetation indices were statistically significant or highly significant at milk ripe stage. In addition, the highest correlation coefficient was above 0.9, suggesting spectral vegetation indices and wheat leaf nitrogen content have high correlation.

3.3. The Establishment of Leaf Nitrogen Content Inversion Equation at Different Growth Stages

At starting stage, wheat grows from prostrate to erect, and the elongation starts with the first internode, but it does not reached out of the ground. The wheat will then begin a vigorous growth stage. Management of fertilizer is crucial for the growth of wheat in this stage. From Table III, it is clear that vegetation index TCARI can be treated as independent variable in establishing leaf nitrogen inversion equation with multiple correlation coefficient at 0.521 for Zhongyou 9507 at the starting stage, whereas Jingdong 8 will has more vegetation indices as independent variables for the leaf nitrogen inversion equation. The regression equation of the maximum multiple correlation coefficient was selected as leaf nitrogen inversion equation. The result showed that the independent variable was NDVI and the multiple correlation coefficient was 0.502.

There is great influence on wheat ears rate and grain seed numbers at jointing stage. While Zhongyou 9507 did not establish significance the inversion equation, Jingdong 8 regarded vegetation index PPR as an independent variable, and established the leaf nitrogen inversion equation with multiple correlation coefficient at 0.589.

Table I. Selected vegetation index.

Abbreviation	Index name	Index formula	References
NRI	Nitrogen reflective index	$NRI = (R_{570} - R_{670}) / (R_{570} + R_{670})$	[12]
PPR	Plant pigment ratio	$PPR = (R_{550} - R_{450}) / (R_{550} + R_{450})$	[13]
GNDVI	Greenness normalized difference vegetation index	$GNDVI = (R_{750} - R_{550}) / (R_{750} + R_{550})$	[14]
OSAVI	Optimization of soil adjusted vegetation index	$OSAVI = (1 + 0.16)(R_{800} - R_{670}) / (R_{800} - R_{670} + 0.16)$	[15]
DVI	Difference vegetation index	$DVI = R_{NIR} - R_{RED}$	[16]
TCARI	Transformed chlorophyll absorption in reflectance index	$TCARI = 3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})(R_{700}/R_{670})]$	[17]
SIPI	Structured independent pigment index	$SIPI = (R_{800} - R_{445}) / (R_{800} - R_{680})$	[18]
SAVI	Soil adjusted vegetation index	$SAVI = [(1 + L)(R_{NIR} - R_{RED})] / (R_{NIR} + R_{RED} + L), L = 0.5$	[19]
RVI	Ratio vegetation index	$RVI = NIR/RED$	[16]
NDVI	Normalized difference vegetation index	$NDVI = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED})$	[20]
PRI	Photochemical reflectance index	$PRI = (R_{570} - R_{531}) / (R_{570} + R_{531})$	[18]

Notes: RNIR and RRED represent infrared and red band spectral reflectance, R_i said spectral reflectance at the wavelength i .

Table II. Correlation coefficient between leaf nitrogen content and spectral vegetation indices at different growth stages for Zhongyou 9507 and Jingdong 8.

	Spectral characteristic Parameters	Starting stage N%	Jointing stage N%	Booting stage N%	Blossom stage N%	Filling stage N%	Milk rip stage N%	
9507	NRI	-0.075	-0.347	0.184	0.036	-0.431	0.778**	
	PPR	-0.445	-0.422	0.136	-0.221	-0.578*	0.511*	
	GNDVI	0.128	-0.338	0.382	0.221	-0.422	0.748**	
	OSAVI	-0.077	-0.139	0.234	0.111	-0.139	0.838**	
	DVI	-0.059	-0.124	0.223	0.098	-0.189	0.801**	
	TCARI	-0.526*	-0.0477	0.078	-0.245	0.169	0.472	
	SIPI	0.033	-0.364	0.298	0.118	-0.44	0.848**	
	SAVI	0.041	-0.347	0.288	0.129	-0.408	0.857**	
	RVI	-0.13	-0.024	0.209	0.087	-0.074	0.499*	
	NDVI	0.043	-0.351	0.288	0.128	-0.414	0.856**	
	PRI	-0.097	0.303	-0.199	-0.161	0.277	-0.517*	
	Jingdong 8	NRI	0.670**	-0.145	-0.085	-0.345	-0.061	0.941**
		PPR	-0.005	-0.591*	-0.252	-0.703**	-0.542*	-0.445
		GNDVI	0.696**	-0.389	0.142	-0.3	0.037	0.926**
OSAVI		0.611*	0.261	-0.067	-0.076	0.043	0.837**	
DVI		0.570*	0.252	-0.096	-0.063	-0.01	0.819**	
TCARI		0.266	0.284	-0.174	0.128	-0.181	-0.088	
SIPI		0.683**	-0.422	-0.004	-0.327	0.016	0.937**	
SAVI		0.708**	-0.288	0.024	-0.27	0.054	0.941**	
RVI		0.451	0.315	-0.106	-0.029	-0.032	0.348	
NDVI		0.709**	-0.303	0.029	-0.276	0.055	0.942**	
PRI		-0.709**	-0.052	-0.108	-0.041	-0.094	-0.962**	

RESEARCH ARTICLE

At blossom stage, more than 50% floret in the upper of wheat opened and anthers spread pollen. At this stage, Zhongyou 9507 did not establish the inversion equation successfully. Jingdong 8 again regarded vegetation index PPR as an independent variable, and established the leaf nitrogen inversion equation at 0.572.

The filling stage is important to seize high yield. Previous research showed that the right amount of nitrogen could significantly increase the grain protein content and improve the grain quality at the filling stage.^{21,22} In this study, both Zhongyou 9507 and the Jingdong 8 could establish the leaf nitrogen inversion equation with vegetation index PPR as an independent variable, and the multiple correlation coefficients were 0.62 and 0.53 respectively.

Milk ripe stage begins after about 10 days of blossom. Seeds start to precipitate starch, and endosperm begins to condensed milk. Zhongyou 9507 selected vegetation index NRI as an independent variable and the multiple correlation coefficient of the equation was 0.85. Whereas Jingdong 8 selected PRI as an independent variable and the multiple correlation coefficient of the equation was 0.925. At milk ripe stage, both regression equations had high correlation coefficients, suggesting that they could well predict wheat leaf nitrogen content.

3.4. Relationship Between Leaf Nitrogen and Grain Protein Content at Grain Filling Stage

The continuous improvement of living condition in China increases the demand of high grain production and most important high quality of food.²³ The indices of grain

quality and flour quality include hardness, protein content, dry gluten, sedimentation value and so on.

Wang et al. analyzed the relationship between wheat leaf nitrogen and grain quality in blossom stage, and established a regression equation of high multiple correlation coefficient. At wheat growth, leaf nitrogen content varies greatly in different parts of wheat at filling stage. The N content on the top of the plant is more closely related with grain quality. Thus, the prediction accuracy may be improved if we concentrate on the top of plant.¹⁰ The result shows that the correlation coefficient between wheat leaf nitrogen content and grain quality indices. Protein and dry gluten have higher correlations with leaf nitrogen content, suggesting that leaf nitrogen content could predict protein and dry gluten. Hardness showed no correlation.

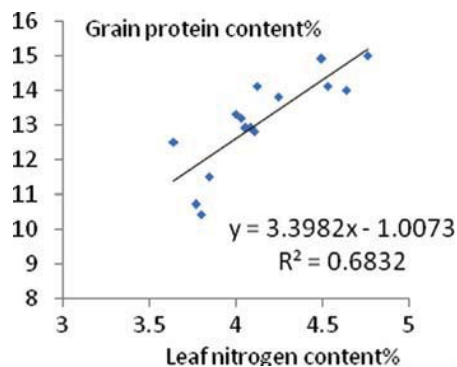
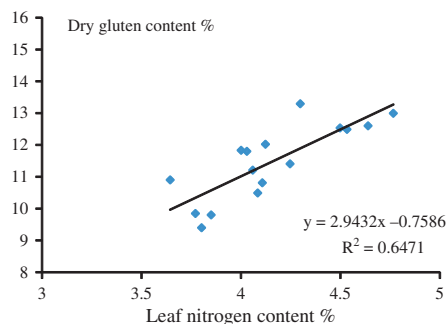
The regression equations between leaf nitrogen and protein or dry gluten were established (Figs. 2 and 3). Both regression equations showed high correlation coefficients. Ten testing samples were selected to estimate protein content and dry gluten content using leaf nitrogen content. The results showed that the root mean square error of the protein estimation was 0.864 and the prediction relative error was 6.03%. For dry gluten content, the RMSE of the estimation was 0.709 and the PRE was 5.737%. These results suggest that these two inversion models have certain reliability in predicting of protein and dry gluten contents.

Jingdong 8 selected PRI as an independent variable and the multiple correlation coefficient of the equation was 0.925. At milk ripe stage, both coefficient of the equation was 0.85. Whereas regression equations had high correlation.

Table III. The regression equation between leaf nitrogen concentration and spectral vegetation index at different growth stages for Zhongyou 9507 and Jingdong 8.

	Growth stage	Independent variable (x)	Dependent variable (y)	Regression equation $y = ax + b$	Multiple correlation coefficient (R^2)	
9507	Starting stage	NRI	Leaf nitrogen	$y = -0.427x + 9.118$	0.521	
	Filling stage	PPR	Leaf nitrogen	$y = -5.28x + 6.095$	0.62	
	Milk ripe stage	GNDVI	Leaf nitrogen	$y = 3.868x + 2.500$	0.85	
		OSAVI	Leaf nitrogen	$y = 9.13x - 0.982$	0.581	
		DVI	Leaf nitrogen	$y = 10.079x - 2.662$	0.559	
		TCARI	Leaf nitrogen	$y = 211.65x - 240.13$	0.703	
		SIPI	Leaf nitrogen	$y = 0.1778x - 0.016$	0.641	
		SAVI	Leaf nitrogen	$y = 6.304x - 1.719$	0.791	
		RVI	Leaf nitrogen	$y = 2.617x + 0.431$	0.812	
		NDVI	Leaf nitrogen	$y = 38.062x + 0.517$	0.297	
		PRI	Leaf nitrogen	$y = 3.834x + 0.444$	0.808	
		NRI	Leaf nitrogen	$y = -7.864x + 3.472$	0.202	
		Jingdong 8	Starting stage	NRI	Leaf nitrogen	$y = 4.121x + 4.5621$
	GNDVI			Leaf nitrogen	$y = 6.04x + 0.836$	0.485
OSAVI	Leaf nitrogen			$y = 365.06x - 416.27$	0.374	
DVI	Leaf nitrogen			$y = 0.058x + 3.191$	0.325	
SIPI	Leaf nitrogen			$y = 6.101x + 0.044$	0.467	
SAVI	Leaf nitrogen			$y = 2.909x + 1.67$	0.501	
NDVI	Leaf nitrogen			$y = 4.335x + 1.652$	0.502	
PRI	Leaf nitrogen			$y = -15.459x + 5.65$	0.501	
PPR	Leaf nitrogen			$y = -12.088x + 8.952$	0.589	
PPR	Leaf nitrogen			$y = 1.709x - 0.345$	0.572	
PPR	Leaf nitrogen			$y = -11.444x + 7.802$	0.530	
Jointing stage	NRI			Leaf nitrogen	$y = 8.581x + 1.778$	0.885
	GNDVI			Leaf nitrogen	$y = 9.428x - 2.661$	0.857
	OSAVI			Leaf nitrogen	$y = 240.62x - 273.73$	0.701
	SIPI		Leaf nitrogen	$y = 9.089x - 4.041$	0.878	
	SAVI		Leaf nitrogen	$y = 3.729x - 0.954$	0.886	
	NDVI		Leaf nitrogen	$y = 5.478x - 0.948$	0.887	
	PRI		Leaf nitrogen	$y = -24.644x + 4.416$	0.925	

The regression equations between leaf nitrogen and protein or dry gluten were established (Figs. 2 and 3). Both regression equations showed high correlation coefficients. Ten testing samples were selected to estimate protein content and dry gluten content using leaf nitrogen content. These results suggest that these two inversion models have certain reliability in predicting of protein and dry gluten contents.

**Fig. 2.** Relationship between wheat leaf.**Fig. 3.** Relationship between wheat leaf.

4. CONCLUSION

This paper studied the relationship between wheat leaf nitrogen content and spectral vegetation index at different growth stages, and established spectral vegetation indices-leaf nitrogen inversion model for two varieties of wheat, whose protein contents were greatly different. The result showed drastic differences between leaf nitrogen content and vegetation indices in wheat. The 11 selected spectral vegetation indices could not consistent related to leaf nitrogen content at all the growth stages. At milk ripe

stage, leaf nitrogen and the most selected spectral vegetation indices exhibited high correlations, and therefore high reliabilities.

Combined with related published papers, we firstly used the spectral vegetation indices and forecasted leaf nitrogen content. Then we correlated leaf nitrogen content with grain hardness, protein content, dry gluten content and sedimentation value. It is concluded that grain quality can be predicted by spectral vegetation indices, particularly using leaf nitrogen contents at filling stage to predict wheat grain protein and dry gluten content. However, this study involved only two wheat varieties and few sample points. Future studies are needed about building models using various types of qualities and large number of sample points in order to improve the model reliability and practical usefulness.

Acknowledgment: This work was subsidized by the Major State Basic Research Development Program of China (2010CB950603), National Natural Science Foundation of China (41071276, 41271412), Hundred Talent Program of the Chinese Academy of Sciences of Wenjiang Huang. The authors are grateful to Mr. Weiguo Li, Mrs. Hong Chang and Zhihong Ma for data collection.

References and Notes

1. W. Zhijie, China Agricultural University Ph.D Dissertation (2004).
2. H. Jingfeng, W. Fumin, and W. Xiuzhen, Zhejiang University Press, Hangzhou (2010), Vol. 1, p. 2.
3. L. Yande, P. Yanying, D. Xiaoling, S. Xudong, and O. Aiguo, *Sensor Letters* 1164, 1169 (2011).
4. W. Jihua, W. Zhijie, H. Wenjiang, M. Zhihong, L. Liangyun, and Z. Chunjiang, *Journal of Remote Sensing* 309, 316 (2004).
5. L. Xiao-li, L. Ming-bo, L. Jia, and T. Yan-lin, *Agricultural Science and Technology* 168, 170 (2011).
6. T. Qiang, L. Shaokun, W. Keru, X. Ruizhi, C. Bin, W. Fangyong, D. Wanying, and X. Chunhua, *Spectroscopy and Spectral Analysis* 3061, 3066 (2010).
7. C. Pengfei, W. Jishun, P. Peng, X. Yuyue, and Y. Ling, *Transactions of the Chinese Society of Agricultural Engineering* 75, 80 (2011).
8. Y. Feng, F. Yamin, L. Jianlong, Q. Yurong, W. Yan, and Z. Jie, *Transactions of the Chinese Society of Agricultural Engineering* 237, 243 (2010).
9. Edited by China Standardization Press, China Standardization Press, Beijing (1998).
10. W. Jihua, H. Wenjing, Z. Chunjiang, Y. Minhua, and W. Zhijie, *Journal of Remote Sensing* 277, 284 (2003).
11. L. Liangyun, *Chinese Academy of Sciences* 65 (2002).
12. T. D. Schleicher, W. C. Bausch, J. A. Delgado, and P. D. Ayers, ASAE Annual International Meeting, St. Joseph, MI, USA (2001), pp. 01–1151.
13. J. Verdebout, S. Jacquemoud, and G. Schmuck, *Optical Properties of Leaves* 169, 191 (1994).
14. A. Gitelson and M. N. Merzlyak, *Journal of Plant Physiology* 494, 500 (1996).
15. G. Rondeaux, M. Steven, and F. Baret, *Remote Sensing of Environment* 95, 107 (1996).
16. C. F. Jordan, *Ecology* 663, 666 (1969).
17. C. S. T. Daughtry, C. L. Walthall, M. S. Kim, C. E. Brown, and J. E. Mc Murtrey, *Remote Sensing of Environment* 229, 239 (2000).
18. J. Peñuelas, I. Filella, and J. A. Gamon, *New Phytologist* 291, 296 (1995).
19. A. R. Huete, *Remote Sensing of Environment* 295, 309 (1998).
20. J. W. Rouse, R. H. Haas, J. A. Schell, D. W. Deering, and J. C. Harlan, NASA/GSFC, Type III, Final Report, Greenbelt, MD, USA (1974), Vol. 1, p. 371.
21. Z. Baojun and J. Jiyun, *Acta Agriculturae Boreali-Occidentalis sinica* 40, 42 (1996).
22. Q. Jing, W. X. Cao, and T. B. Dai, *Oecologia* 463, 474 (1992).
23. W. Jihua, Z. Chunjiang, H. Wenjing, Beijing, Science Press (2008), Vol. 290, p. 294.