INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–753/2014/16–3–498–504
http://www.fspublishers.org

Full Length Article

Estimation of Grain Protein Content in Winter Wheat by Using Three Methods with Hyperspectral Data

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Abstract

Grain protein content (GPC) is an important quality indicator for cereal crops to meet variety of needs of commodity. The objectives of this study were: 1) to analyze relationships between the single vegetation indexes and GPC; 2) to improve estimation accuracy of GPC by using two or three vegetation indexes and partial least squares method (PLS); 3) to compare the performance of the proposed models by the three methods. Vegetation indexes and concurrent GPC of samples were selected in Xiaotangshan experimental sites, Beijing, China, during 2008/2009, 2009/2010 and 2011/2012 winter wheat growth seasons. This study showed that the GPC could be effectively estimated using three methods (single vegetation indexes, two or three vegetation indexes and PLS). The lowest RMSE and highest R^2 were $PLS_{(b)}$ regression model for PLS (R^2=0.63 and RMSE=0.615%); SIPI, OSAVI and MTVI2 for three vegetation indexes (R^2=0.57 and RMSE=0.84%); MTVI2 and MTCI for two vegetation indexes (R^2=0.56 and RMSE=0.92%) and DCNI I for single vegetation indexes (R^2= 0.53 and RMSE=1.12%), respectively. The $PLS_{(b)}$ was better than others methods for estimating GPC in winter wheat. But two or three vegetation indexes also has its own merit, particularly when taking into consideration the simplicity of its application. This method may be provide guideline for improving the estimation accuracy of winter wheat GPC in a large worldwide by using different algorithm and satellite images data. © 2014 Friends Science Publishers

Keywords: Grain protein content; Vegetation index; Partial least squares method; Winter wheat

Introduction

Wheat is a very important grain, as it is one of China's leading crops, up there with rice, corn, etc. Grain protein content (GPC) is a key indicator for wheat quality. In general, the protein content of wheat grain ranges from 9-18%, and the low and high protein content should be used directly to determine usage and commodity value (Li *et al*., 2008). The current traditional methods of GPC determination completed in the laboratory, and it takes a lot of manpower and time for scientists to get GPC results. Some studies that the nitrogen (N) concentration is strongly related with GPC in winter wheat (*Triticum aestivum* L.) plants (Wang *et al*., 2003b; Tian, *et al*., 2004; Shi *et al*., 2005; 2010). Therefore, a method that would allow real-time, in-field detection of crop nitrogen needs was to improve the field nitrogen management strategy and predict the protein content of wheat before harvest could be useful in wheat crops.

With development of remote sensing has provided new tools for chlorophyll and nitrogen content estimation at grain crops (Jin *et al*., 2012; 2013a, b) and reduced labor and material costs on agriculture practices, and then it would lay a foundation for GPC estimation in wheat plants in a large worldwide. Therefore, some authors have studied the relationship between vegetation indexes and GPC and attempted to estimate quantitatively GPC. Wang *et al.* (2003a; 2004) used the sensitive wavebands of chlorophyll and plant pigment ratio (PPR) were used to estimate GPC, the results showed that the characteristic wavebands and vegetation index was highly related with GPC. The results of Huang *et al*. (2004) suggested that it was available to use the structure insensitive pigment index (SIPI) for estimating nitrogen content and protein content of winter wheat at anthesis. Tian *et al*. (2004) studied the relationships between chlorophyll meter values (SPAD values), canopy reflectance and GPC; these results demonstrated that the characteristic wavebands of GPC were 610, 710, 950 and 1500 nm, respectively. But Li *et al*. (2005) suggested that the characteristic wavebands of GPC were 445, 660, 680, 710, 950, 1220, 1500, 1100 and 1650 nm, respectively, Lu *et al.* (2006) and Xiao *et al*. (2007) indicated that the characteristic wavebands of GPC were 670 and 890 nm, respectively. Feng *et al.* (2008) studied relationships

To cite this paper: Jin, X.L., X.G. Xu, H.K. Feng, X.Y. Song, Q. Wang, J.H. Wang, W.S. Guo, 2014. Estimation of grain protein content in winter wheat by using three methods with hyperspectral data. *Int. J. Agric. Biol.,* 16: 498–504

between the first derivative spectrum at 742 nm (FD742) and GPC, and the results indicated the FD742 could be used to estimate GPC for winter wheat. Landsat TM images are used to build green degree vegetation index (VIgreen, derived from the canopy spectral reflectance at green and red bands) at anthesis, the results demonstrated that VIgreen was highly significantly correlated with the final grain protein content (determination coefficient, R^2= 0.46). Three Landsat TM satellite images and three Envisat Advanced Synthetic Aperture Radar (ASAR) satellite images were successfully used to monitor crop condition and forecast grain yield and protein content (Liu *et al*., 2006), and it indicated that a good relationship between the Structure Insensitive Pigment Index (SIPI) and the protein content of winter wheat. Reyniers *et al*. (2006) used the Crops can spectrum instruments and a Color Infrared (CIR) aerial image (KODAK CIR 2443) to estimate the GPC of winter wheat and estimation accuracy reached 90% at one month before harvest. Pettersson and Eckersten (2007) estimated grain protein in spring malting barley grown in northern Europe, it suggested that the Transformed Chlorophyll in Absorption Reflectance Index (TCARI) was highly related to the protein content for a specific cultivar (R^2=0.78). Li *et al*. (2008) predicted protein content in winter wheat based on Landsat TM images and nitrogen accumulation, the results indicated that the predicted values were consistent with the measured values, the root mean square error (RMSEs) were from 0.47% to 0.59%. Chen *et al*. (2011) detected wheat grain protein content by using nitrogen nutrition index (NNI) and vegetation indexes. Jin *et al*. (2013c) also tried to estimate the GPC of winter wheat based on the new indexes. So far, some studies estimated grain protein content (GPC) by using the different characteristic wavebands and vegetation indexes. Based on previous studies on relationships between vegetation indexes and GPC the objectives of this study were to: 1) analyze relationships between single vegetation index and GPC; 2) improve estimation accuracy of GPC by using two or three vegetation indexes and partial least squares method (PLS); 3) compare the three methods. This method could be providing a theoretical basis and reference for GPC estimation in winter wheat.

Materials and Methods

Design of Experiment

The experiment was conducted in the national experimental station for precision agriculture, located at Xiaotangshan, Changping district, Beijing (40°10′31″ N to 40°11′18″N, 116°26′10″ E to 116°27′05″ E) across 2008/2009, 2009/2010 and 2011/2012. The soil was a silt clay loam with organic matter 1.58-2.00%, total N 0.10-0.12%, Nitrate (N) 3.16-14.82 mg kg^{-1}, available phosphorus 3.14-21.18 mg kg^{-1} and available potassium 86.83-120.62 mg kg^{-1} in the 0-30 cm layer.

Experiment one: The experiment included three sowing dates, three winter wheat varieties and four nitrogen levels in 2008/2009. Sowing dates: 9/28, 10/7 and 10/20 in 2008, respectively. There local wheat cultivars: Nongda195, Jingdong8, and Jing9428 were planted. Nitrogen fertilizer as urea was applied at four rates (45, 98, 150, and 203 kg N ha^{-1}) before planting at 9/28; the N application was distributed in three splits: 50% at seeding, 25% at jointing, and 25% at heading. For all treatments, 82.5 kg ha^{-1} P_2O_5 (as monocalcium phosphate [$Ca(H_2PO_4)_2$]), and 75 kg ha^{-1} K_2O (as KCl), were applied prior to seeding, were applied prior to seeding. The plots were 100 m^2 in 2008/2009. The experiment was a 2-way factorial arrangement of treatments in a randomized complete block design with three replications for each treatment. Other management elements followed local standard practices of wheat production.

Experiment two: The experiment included three sowing dates, three winter wheat varieties and four nitrogen levels in 2009/2010. Sowing dates: 9/25, 10/5 and 10/15 in 2009, respectively. There local wheat cultivars: Nongda195, Jingdong13 and Jing9428 were planted. Nitrogen fertilizer as urea was applied at four rates (56, 78, 109, and 165 kg N ha^{-1}) before planting at 9/25. The plot was 100 m^2 in 2009/2010. Remaining will be same as above.

Experiment three: Four local wheat cultivars: Nongda211, Zhongmai175, Jingdong8, and Jing9843 were planted on 9/25, 2011. The plot was 300 m^2 in 2011/2012. Remaining will be same as above.

Measurement of Canopy Reflectance

Spectral measurements were carried out form anthesis stages to filling stages, and respective dates: 12th May, 2009, 19th May, 2010, and 10th May, 2012. All canopy spectral measurements were held in a nadir orientation 1.3 m above the canopy. Measurements took place under clear sky conditions between 10:00 and 14:00 Beijing local time. Measurements were taken using an ASD Field Spec Pro Spectrometer (Analytical Spectral Devices, Boulder, CO, USA). This spectrometer was fitted with a 25° field of view fiber optic operating in the 350-2500 nm spectral region, with a sampling interval of 1.4 nm between 350 and 1050 nm, and a sampling interval of 2 nm between 1050 and 2500 nm There was a spectral resolution of 3 nm at 700 nm, and of 10 nm at 1400 nm A 40 cm × 40 cm $BaSO_4$ calibration panel was used for calculating the black and baseline reflectance. To reduce the possible effects of sky and field conditions, spectral measurements were taken at four sites in each plot and then averaged to represent the canopy reflectance of each plot. Vegetation radiance measurement was taken by averaging 10 scans at an optimized integration time, with a dark current correction at every spectral measurement. A panel radiance measurement was taken before and after the vegetation measurement by two scans each time.

Plant Measurement

Dry biomass weight: The biomass of the spectral positions was collected, and aboveground biomass was destructively sampled by randomly cutting 0.375 m^2 vegetation in each plot from the scanned plants. All plant samples were heated to 105°C, oven dried at 85°C to constant weight, and then weighed.

Chlorophyll content: Some leaves were collected using a 0.4 cm diameter hole puncher. Then about 0.2 g of each sample was punched off in the laboratory. Selected samples were placed in 95% ethanol or acetone solution and then left to stand for 24 h in the dark. After the 24 h treatment the leaves were white-green in color. Finally, leaf pigment densities were measured using a colorimetric spectrophotometer.

Nitrogen content: Dry plant material was then ground to pass through a 240-mesh screen, and analyzed for total N using a Carlo-Erba NA 1500 dry combustion analyzer (Carlo Erba, Milan, Italy) (Schepers *et al*., 1989).

Leaf area index (LAI): The leaf area index (LAI) was measured using the LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE, USA) with spectrometric measurements at the same position.

Plant water measurement: Collected plant samples were placed in a paper bag, sealed in a plastic bag, and placed in a cool dark container in order to avoid as much water loss as possible. Upon return from the field, leaves and stems were separated and weighed. Then, All plant samples were heated to 105°C, oven dried at 65°C to constant weight, and then weighed. Leaf water content (LWC) was calculated as follows:

$$LWC=(LFWC-LDWC)/LFWC \times 100\% \quad (1)$$

Where LFWC is the sample fresh leaf weight (kg), and LDWC is the sample dry leaf weight (kg).

Protein content: The grain of winter wheat was harvest and then threshed. Grain protein content (GPC) was measured by using FOSS InfratecTM 1241 Grain Analyzer (Tecator, Hoganas, Sweden), and the unit of GPC is percent sign (%).

Partial Least Squares Method

Partial least squares regression is an extension of the multiple linear regression models (e.g., Multiple Regression or General Stepwise Regression). This method is particularly useful when one needs to predict a set of dependent variables from a (very) large set of independent variables. In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable Y, and a set of predictor variables, the X variables, so that

$$Y = b0 + b1X1 + b2X2 + ... + bpXp \quad (2)$$

In this equation, b0 is the regression coefficient for the intercept, and the bi values are regression coefficients (for variables 1 through p) computed from the data.

Statistical Analysis

The correlations between the grain protein content (GPC) data and the vegetation index were analyzed using SPSS software (16.0, SPSS, Chicago, IBM, USA) and Matelab software version 7.4.0 (release R2007a). The coefficient of determination (R^2) and root mean square error (RMSE) were used to quantify the amount of variation explained by the relationships developed, as well as the accuracy of the developed relationships. Generally, the performance of the model was estimated by comparing the differences in prediction of the R^2 and RMSE. The higher the R^2 was and the lower the RMSE value was, the higher the precision and accuracy of model to predict winter wheat GPC were.

Vegetation Indexes Selection

Based on published literatures, thirteen vegetation indexes, which are good relationships between leaf chlorophyll content and chlorophyll vegetation indexes (CVIs) were used (Table 1).

Results

Single Vegetation Indexes and Grain Protein Content (GPC)

Relationships between Grain protein content (GPC) and vegetation indexes were significant with the exception of NDVI and OSAVI. The MTVI2, MCARI, MCARI/MTVI2, TCARI, TCARI/OSAVI, SIPI and PPR were significantly negatively correlated with GPC, and the correlation coefficient (r) of values were -0.42, -0.65, -0.66, -0.71, -0.71, -0.29 and -0.67, respectively (Table 2). The remaining were positively correlated, of which the DCNI I had the highest r of 0.73. The results show that the r of $CI_{red\ edge}$, MTCI and RENDVI were 0.56, 0.70 and 0.50, respectively. This suggested that most of vegetation indexes could be used to estimate GPC in winter wheat. To further refine the relationships between GPC and vegetation indexes, linear and nonlinear regression analysis was done using these vegetation indexes as independent variables. Of the determination coefficients (R^2): nine were below 0.5, five were above 0.7 in all vegetation indexes. The lowest and highest R^2 were NDVI and DCNI I, with R^2 values of 0.02 and 0.53. R^2 behaved similarly to r, and had a significant correlation with GPC. But there were differences in the highly significant correlation order between R^2 and r (Table 2). Fifty-six pairs of data were used to establish the regression models in 2008/2009 and 2009/2010 for estimating GPC in winter wheat (Table 2). The lowest and highest regression models were DCNI I and NDVI according to the significant correlation. The $CI_{red\ edge}$, MCARI, TCARI, OSAVI, DCNI I, MTCI, RENDVI, PPR and NDVI were fitted to power regression models, the MTVI2, MCARI/MTVI2, TCARI/OSAVI and SIPI were fitted to exponential equations.

Table 1: Summary of vegetation indices studied for grain protein content (GPC)

Index	Name	Formula	Developer(s)
$CI_{red\ edge}$	Red edge model	$(R_{750}/R_{720})-1$	Gitelson *et al*. (2005)
MTVI2#	Modified triangular vegetation index 2#	$1.5(1.2(R_{800}-R_{550})-2.5(R_{670}-R_{550}))/\mathrm{sqrt}((2R_{800}+1)^2-(6R_{800}-5\mathrm{sqrt}(R_{670}))-0.5)$	Broge and Leblanc. (2000)
MCARI	Modified chlorophyll absorption ratio index	$(R_{700}-R_{670}-0.2(R_{700}-R_{550}))(R_{700}/R_{670})$	Daughtry *et al*. (2000)
MCARI/MTVI2	Combined index I#	MCARI/MTVI2	Eitel *et al*. (2007)
TCARI	Transformed chlorophyll absorption in reflectance index	$3((R_{700}-R_{670})-0.2(R_{700}-R_{550})(R_{700}/R_{670}))$	Haboudane *et al*. (2002)
OSAVI	Optimized soil-adjusted vegetation index	1.16(R800–R670)/(R800+R670+0.16)	Rondeaux *et al*. (1996)
TCARI/OSAVI	Combined Index II#	TCARI/OSAVI	Haboudane *et al*. (2002)
DCNI I#	Double-peak canopy nitrogen index I	$(R_{720}-R_{700})/(R_{700}-R_{670})/(R_{720}-R_{670}+0.03)$	Chen *et al*. (2010)
MTCI	MERIS terrestrial chlorophyll index	$(R_{750}-R_{710})/(R_{710}-R_{680})$	Dash and Curran. (2004)
RENDVI	Red edge Normalized difference vegetation index	$(R_{750}-R_{710})/(R_{750}+R_{710})$	Gitelson and Merzlyak (1996)
SIPI	Structure insensitive pigment index	$(R_{800}-R_{445})/(R_{800}-R_{680})$	Penuelas *et al*. (1995)
PPR	Plant pigment ratio	$(R_{550}-R_{450})/(R_{550}+R_{450})$	Metternicht (2003)
NDVI	Normalized difference vegetation index	$(R_{800}-R_{670})/(R_{800}+R_{670})$	Rouse *et al*. (1974)

# Denotes named by this study; Di denotes derivative reflectance at band i (nanometer); Ri denotes reflectance at band i (nanometer).

Table 2: Relationship between grain protein content (GPC) and single vegetation indexes in winter wheat. (n=56)

Vegetation index	Correlation coefficient (r)	Regression model	Determination coefficient (R^2)
$CI_{red\ edge}$	0.56**	$y = 14.49x^{0.249}$	0.32**
MTVI2#	-0.42**	$y = 20.92e^{-0.34x}$	0.19**
MCARI	-0.65**	$y = 24.81x^{-0.33}$	0.38**
MCARI/MTVI2	-0.66**	$y = 21.35e^{-0.06x}$	0.41**
TCARI	-0.71**	$y = 39.71x^{-0.50}$	0.51**
OSAVI	$0.07^{N.S.}$	$y = 15.57x^{0.301}$	$0.03^{N.S.}$
TCARI/OSAVI	-0.71**	$y = 21.62e^{-0.04x}$	0.52**
DCNI I#	0.73**	$y = 23.49x^{0.376}$	0.53**
MTCI	0.70**	$y = 21.92x^{1.482}$	0.50**
RENDVI	0.50**	$y = 19.89x^{0.425}$	0.27**
SIPI	-0.29*	$y = 79.89e^{-1.60x}$	0.10*
PPR	-0.67**	$y = 8.230x^{-0.60}$	0.42**
NDVI	$0.06^{N.S.}$	$y = 16.25x^{0.297}$	$0.02^{N.S.}$

Note: **,* and N.S. represent significant at the 0.01 and 0.05 levels of probability, and not significant respectively.

Two or Three Vegetation Indexes and GPC

To improve the GPC estimation accuracy for winter wheat, we studied relationships the two or three vegetation indexes and GPC. The results showed that the two vegetation indexes (NDVI and RENDVI, NDVI and MTCI, OSAVI and RENDVI,OSAVI and MTCI and MTVI2 and MTCI) could be used to further increase the GPC estimation accuracy (Table 3). The highly significant correlation order of R^2 was: MTVI2 and MTCI, NDVI and MTCI, OSAVI and MTCI, OSAVI and RENDVI and NDVI and RENDVI. Similarly, three vegetation indexes (NDVI, SIPI and MTVI; NDVI, RENDVI and $CI_{red\ edge}$; NDVI, MTCI and MCARI; SIPI, RENDVI and MTVI2; SIPI, OSAVI and MTVI2) were also increased the GPC estimation accuracy (Table 4), the highly significant correlation order of R^2 was: SIPI, OSAVI and MTVI2; NDVI, SIPI and MTVI; SIPI, RENDVI and MTVI2; NDVI, MTCI and MCARI; NDVI, RENDVI and $CI_{red\ edge}$. Compared single vegetation indexes and two or three vegetation indexes, the best regression models in single vegetation indexes, two or three vegetation indexes were estimated with R^2 from 2008/2009 and 2009/2010 data and RMSE by using data from 2011/2012 data. These results showed that two vegetation indexes (MTVI2 and MTCI, R^2=0.56 and RMSE=0.92%) (Fig. 1b) and three vegetation indexes (SIPI, OSAVI and MTVI2 R^2=0.57 and RMSE=0.84%) (Fig. 1c) were better than single vegetation indexes (DCNI, R^2=0.53 and RMSE=1.12%) (Fig. 1a). The results indicated that two vegetation indexes and three vegetation indexes could be used to improve the GPC estimation accuracy in winter wheat.

Partial Least Squares (PLS) and GPC

To study the relationship between GPC and partial least squares method (PLS), we used data from 2008/2009 and 2009/2010 to establish the regression models of GPC, and then data from 2011/2012 was validated the regression models of GPC. The results showed that the $PLS_{(b)}$ of regression model (R^2=0.63) were better than the $PLS_{(a)}$ of regression model (R^2=0.62) for estimating GPC (Table 5), but the RMSE of $PLS_{(b)}$ regression model (RMSE=0.615%) was close to the RMSE of $PLS_{(a)}$ regression model (RMSE=0.617%) (Figs. 2a and 2b). The results indicated that the increased PPR, DCNI, TCARI, TCARI/OSAVI and MCARI/MTVI2 were not significantly improved the GPC estimation accuracy based on the $PLS_{(a)}$ regression model.

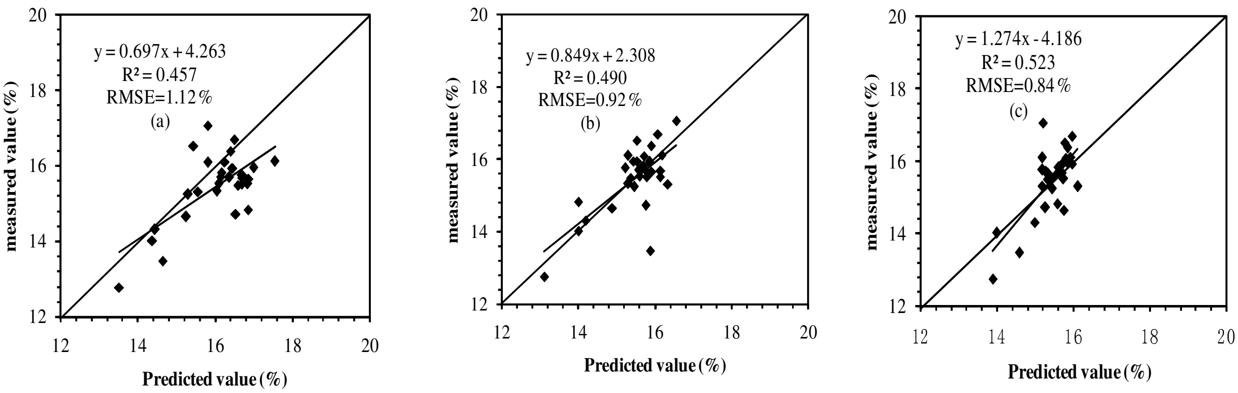

Fig. 1: A comparison between the predicted and the measured values (a) single vegetation indexes (DCNI), (b) two vegetation indexes (MTVI2 and MTCI) and (c) three vegetation indexes (SIPI,OSAVI and MTVI2) for grain protein content (GPC) in winter wheat

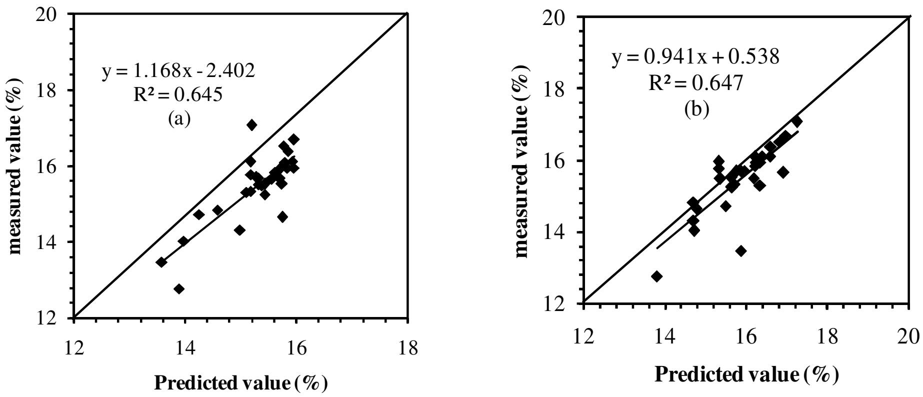

Fig. 2: Relationships between the predicted and the measured values [(a)$PLS_{(a)}$ and (b) $PLS_{(b)}$] for grain protein content (GPC) in winter wheat

The results suggested that the PLS could be also used to further improve the GPC estimation accuracy in winter wheat. The performance of PLS regression model was better than two vegetation indexes and three vegetation indexes (Tables 3, 4 and 5, Figs. 1b, 1c, 2a and 2b.). These results indicated that the PLS regression model was also used for improving the GPC estimation accuracy in winter wheat.

Discussion

Grain protein content (GPC) is an important standard for flour processing enterprises. It is also important to be able to evaluate winter wheat grain quality. Results of this study showed that the GPC could be effectively estimated using single vegetation indexes (MTVI2, MCARI, MCARI/MTVI2, TCARI, TCARI/OSAVI, SIPI, PPR, DCNI, $CI_{red\ edge}$, MTCI and RENDVI) (Table 2), two vegetation indexes (NDVI and RENDVI, NDVI and MTCI, OSAVI and RENDVI,OSAVI and MTCI and MTVI2 and MTCI) (Table 3), three vegetation indexes (NDVI, SIPI and MTVI; NDVI, RENDVI and $CI_{red\ edge}$; NDVI, MTCI and MCARI; SIPI, RENDVI and MTVI2; SIPI, OSAVI and MTVI2) (Table 4) and partial least squares (PLS) (Table 5). Wang *et al.* (2004) showed that the leaf N concentration could be used to predict GPC in winter wheat at anthesis. Some studies reported that chlorophyll reflectance indices based on the chlorophyll absorption bands could be used to estimate wheat crop nitrogen status (Filella *et al*., 1995; Huang *et al*., 2004; Pettersson and Eckersten, 2007; Jin *et al*., 2013c). The results showed that a good relationships between GPC and single vegetation indexes with the exception of NDVI and OSAVI, especially DCNI I, with R^2 and RMSE values of 0.53 and 1.12%, respectively (Table 2). The result was consistent with the result of Chen *et al.* (2010). Because DNCI I included the chlorophyll sensitivity wavebands (670, 700 and 720 nm), and it reduces the effect of LAI, Therefore a high correlation between DCNI I and GPC. The result presented that the performance of two or three vegetation indexes was better than single vegetation indexes for estimating GPC in winter wheat (Tables 2, 3 and 4). It is mainly reason that two or three vegetation indexes included more chlorophyll

Table 3: Relationships between grain protein content (GPC) and two vegetation indexes in winter wheat. (n=56)

Regression model	Correlation coefficient (r)	Determination coefficient (R^2)
y=-52.244×NDVI+49.991×RENDVI+31.65	0.741	0.551
y=-21.652×NDVI+48.892×MTCI-4.918	0.746	0.556
y=-45.438×OSAVI+50.268×RENDVI+31.68	0.742	0.552
y=-18.764×OSAVI+48.973×MTCI-4.987	0.745	0.555
y=-29.234×MTVI2+38.906×MTCI+10.712	0.747	0.558

Table 4: Relationship between grain protein content (GPC) and three vegetation indexes in winter wheat. (n=56)

Regression model	Correlation coefficient (r)	Determination coefficient (R^2)
y=33.904×NDVI-84.464×SIPI-110.003×MTVI2+170.942	0.756	0.571
y=-59.893×NDVI+78.048×RENDVI-5.086×$CI_{red\ edge}$+29.253	0.749	0.562
y=-31.555×NDVI+64.601×MTCI+0.415×MCARI-10.853	0.752	0.565
y=-65.812×SIPI+16.654×RENDVI-70.541×MTVI2+136.111	0.753	0.567
y=-84.643×SIPI+28.995×OSAVI-109.442×MTVI2+170.987	0.756	0.572

Table 5: Grain protein content (GPC) was estimated by using partial least squares method (PLS) in winter wheat

Methods	Input vegetation indexes	R^2	RMSE
$PLS_{(a)}$	RENDVI, MTVI2, MTCI, OSAVI, SIPI, $CI_{red\ edge}$, MCARI and NDVI	0.62	0.617 %
$PLS_{(b)}$	NDVI, SIPI, PPR, RENDVI, RENDVI, MTCI, DCNI, TCARI, OSAVI, TCARI/OSAVI, MCARI, MTVI2, $CI_{red\ edge}$ and MCARI/MTVI2	0.63	0.615 %

sensitivity wavebands than single vegetation indexes, thereby two vegetation indexes were closely related with GPC. Similarly, three vegetation indexes (SIPI, OSAVI and MTVI2; R^2=0.57 and RMSE=0.84%) was better than two vegetation indexes for estimating GPC in winter wheat (MTVI2 and MTCI, R^2=0.56 and RMSE=0.92%) (Table 3 and 4). The results showed that the PLS was used to improve the GPC estimation accuracy (Table 5 and Fig.2). The R^2 and RMSE of $PLS_{(b)}$ regression model were 0.63 and 0.615%, respectively (Table 5 and Fig. 2b), the R^2 and RMSE of $PLS_{(a)}$ regression model were 0.62 and 0.615, respectively (Table 5 and Fig. 2a). Compared with two or three vegetation indexes, the PLS was higher related with GPC. It could be combined the more chlorophyll sensitivity wavebands than two or three vegetation indexes. The results of Nguyen *et al*. (2006) indicated that the estimation accuracy of rice nitrogen content was obviously improved by using PLS regression model. Atzberger *et al*. (2010) demonstrated that the PLS regression model contained the most sensitive wavebands, and it seems therefore much better adapted to deal with potentially confounding factors (such as the soil factor). Similarly, this paper also found the PLS regression model demonstrated a high precision for GPC estimation; this result was in agreement with those of Atzberger *et al*. (2010) and Nguyen *et al*. (2006). The RMSE of $PLS_{(b)}$ regression model (RMSE=0.615%) was close to the RMSE of $PLS_{(a)}$ regression model (RMSE=0.617%) (Figs.2a and 2b), The results suggested that the $PLS_{(b)}$ regression model included some invalid vegetation indexes, which were not used to further improved the GPC estimation accuracy. We will select the most chlorophyll sensitivity wavebands by using some methods (such as genetic algorithms) to input into PLS for improving the estimation accuracy of GPC in winter wheat in future.

In conclusion, this study showed that the GPC could be effectively estimated using the single vegetation indexes, two vegetation indexes, three vegetation indexes and partial least squares (PLS). The lowest RMSE and highest R^2 were DCNI I for single vegetation indexes (R^2= 0.53 and RMSE=1.12%); MTVI2 and MTCI for two vegetation indexes (R^2=0.56 and RMSE=0.92%); SIPI, OSAVI and MTVI2 for three vegetation indexes (R^2=0.57 and RMSE=0.84%); $PLS_{(b)}$ regression model for PLS (R^2=0.63 and RMSE=0.615%). The performance of $PLS_{(b)}$ regression model was better than others methods (single vegetation indexes, two vegetation indexes and three vegetation indexes) for estimating GPC in winter wheat.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No.41171281 and 41271415), Beijing Nova Program (Grant No.2011036), the Training of Innovative Postgraduate Students Program of Jiangsu Province (Grant No. CXZZ12_0904) and China Postdoctoral Science Foundation (2013T60080). The authors are grateful to Mr. Weiguo Li, and Mrs. Hong Chang for data collection.

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(Received 04 June 2013; Accepted 28 October 2013)