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RESPONSE OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) SPECTRAL REFLECTANCE CHARACTERISTICS TO CHLOROPHYLL CONTENT IN PLANTING DENSITIES APPLICATION RATE

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Abstract

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The leaf chlorophyll content of wheat is one of important agronomic parameters to analyze the growth environment and assess the growth status. The non-destructive and rapid monitoring of plant leaf chlorophyll content is of practical importance on the optimization of cultivation in winter wheat. In this study, the canopy spectral characteristics of different winter wheat cultivars planted with different densities (PD) were analyzed, and coefficient of variation (C.V) and first derivative reflectance (FDR) of spectral reflectance were extracted. The sensitive bands for the leaf chlorophyll were determined by means of correlation analysis, and then the quantitative relationship between leaf chlorophyll content and canopy reflectance spectra were established. The results showed that there was a major difference in canopy reflectance spectra among different planting densities. As the density increased reflectance in the region of visible spectrum increased, while in the region of near infrared, reflectance decreased. The reflectance spectra of two cultivars showed the opposite in the region of visible and near infrared spectrum. Near infrared bands (780-1100 nm) was better than visible bands (460-730 nm) in differentiating planting densities of different wheat cultivars. The bands of 650, 670, 1200 and 1260 nm could be defined as sensitive bands to monitor leaf chlorophyll content of winter wheat. The predicting models of leaf chlorophyll content were established with the use of the variables of vegetable index (VI). The testing results showed better effect, with the average deviation of model itself lower than the average deviation of cross test, so leaf chlorophyll content could be well imitated and forecasted by canopy spectral. Overall, the monitoring models of leaf chlorophyll content showed good test results, with reliable estimation from DVI (1200, 670) of "Le639" at the booting stage and PVI (1200, 730) of "Jing9549" at the joining stage. Compared with single variable models, there was a higher R^2, lower SE and RMSE for composite models that revealed better anticipating effect of composite models than single variable models. Therefore, it is feasible to monitor leaf chlorophyll content of winter wheat in the key growth stages using the spectral vegetation indices.

Key words: planting densities, spectral characteristics, leaf chlorophyll content, first derivative reflectance, variation coefficient

Introduction

In recent years, along with the rapid development of remote sensing technology (Lu et al., 2004; Rogana and Chen, 2004), this technology has been more widely used in crop identification (Yafit and Maxim, 2002), growth monitoring (Boken et al., 2002) and yield dynamic estimation (Daughtry et al., 1992; Serrano et al., 2000) and precise fertilization (Tubaña et al., 2011), etc. The leaf chlorophyll content of wheat is one of important agronomic parameters to analyze the growth environment and assess the growth status. Therefore, the non-destructive and rapid monitoring of plant leaf chloro-

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phyll content is of practical importance on the optimization of cultivation in winter wheat. Under different nitrogen levels, chlorophyll a had a significant relationship with the reflectance of 550 nm, 680 nm, and the reflectance of 630 nm is the best indicator to chlorophyll b (Filella et al., 1995; Blackburn et al., 1998a). Relative research showed that the vegetation index, such as Pigment Specific Simple Ratio (PSSR), Pigment Specific Normalized Difference (PSND) (Blackburn, 1998b), Moderate Resolution Imaging Spectroradiometer - Normalized Difference Vegetation Index (MODIS-NDVI) (Rouse et al., 1974), Enhanced Vegetation Index (EVI) (Liu et al., 1995) also had a more significant correlation with leaf chlorophyll content (Blackburn, 1998a; Wu et al., 2010).

In analysis of plant spectral data for extracting characteristic information on target objects many methods have been adopted, including vegetation parameters, derivative algorithms and so on (Filella et al., 1995; Pinar and Curran, 1996; Kochubey and Kazantsev, 2007; Ciganda et al., 2009; Ju et al., 2010; Yi and Anatoly, 2012; Jin et al., 2012). Results showed that red edge (Jago, et al., 1999; Zarco-Tejada and Miller, 2000) had a relative relationship with leaf chlorophyll concentration and chlorophyll density. In Addition, total nitrogen and total chlorophylls content could be evaluated by $d\lambda_{NIRP}$ and $d\lambda_{red}$, respectively (Zhao et al., 2002).

Previous research mostly focused on monitoring chlorophyll content of crop under different nitrogen levels, however, the crop planting density is one of the most important cultural practices (Shafi et al., 2012) and plays an important regulatory role in the utilization of solar energy resources (Nekonam and Razmjoo, 2007). Therefore, it is one of the important adjusting measures to realize high-yield in crops. So far, there are few reports about determining and monitoring the sensitive bands for chlorophyll content under different winter wheat cultivars and different planting densities.

Thus, the objective of the present study was to analyze the spectral response of wheat canopy under different wheat varieties and different planting densities. The sensitive bands to the variation of chlorophyll would be extracted; the quantitative relation between chlorophyll content and the wheat canopy spectral characteristics would also be analyzed. The results would help to provide the theoretical references for field management and non-destructive, rapid detection of chlorophyll content in wheat crop.

Materials and Methods

Experiment design

Experiment 1. The experiment was carried out at the experiment station of Shan Xi Agriculture University (E112°34′16.96″, N37°25′19.81″) area of China for the season of September 2011 to July 2012. The experimental factors included different planting density and different varieties of winter wheat. The experiment with the plot size of 30 m^2 area was a randomized complete block design with 3 replications. For all the treatments, sufficient compound fertilizer was applied at 50 $kg{\cdot}ha^{-1}$ prior to seeding. The gleyed paddy soil contained 22.01 $g{\cdot}kg^{-1}$ organic matter, 53.8 $mg{\cdot}kg^{-1}$ available N, 18.43 $mg{\cdot}kg^{-1}$ available phosphate P (P_2O_5), and 236.9 $mg{\cdot}kg^{-1}$ available potassium (K_2O). Two winter wheat cultivars were selected: “Jing9549” and “Le639”. Five plant densities were applied: 3 million $plant{\cdot}ha^{-1}$ (PD1); 4.5 million $plant{\cdot}ha^{-1}$ (PD2); 6.0 million $plant{\cdot}ha^{-1}$ (PD3); 7.5 million $plant{\cdot}ha^{-1}$ (PD4); 9.0 million $plant{\cdot}ha^{-1}$ (PD5). Other management followed local standard practices in wheat production. The experiment data were used for establishing models.

Experiment 2. The experiment was carried out at the experiment station of Shan Xi Agriculture University from 2010 to 2011. Three winter wheat cultivars were selected: “Jing9549”, “Le639”and “Chang4738”. The soil in the fields was calcareous cinnamon soil with 20.07 $g{\cdot}kg^{-1}$ organic matter, 49.3 $mg{\cdot}kg^{-1}$ available N, 18.79 $g{\cdot}kg^{-1}$ available P, and 247.3 $g{\cdot}kg^{-1}$ available K. The plant densities treatments and management were same with Exp.1. The experiment data were used for testing models.

Measure indicators and methods

All canopy spectral measurements were taken using an MSR-16 spectrometer. This spectrometer is fitted with a 31.1° field of view, operating in the 452-1650 nm (460, 510, 560, 610, 660, 680, 710, 760, 810, 870, 950, 1 100, 1 200, 1 300, 1 500, 1 650 nm) spectral region. Spectrometer was calibrated with a white standard white board. The measurements were carried out from a height of 1.2 m above the canopy (the height of wheat was 75 to 90 cm at maturity) under clear sky conditions between 10:00 h and 14:00 h (Beijing local time, China). Measurements of vegetation radiance were made at 3 sample sites in each plot, with each sample from averaging 5 scans. Canopy spectrum was obtained at several major growth stages, including jointing, booting, heading and grouting stage.

After each measurement of canopy spectral reflectance, an area of 0.25 m^2 (2 rows and 0.5 m long) of wheat plants from each plot was collected for determination of chlorophyll content. From each sample, all top leaf and top second leaf were separated from the stem, and leaf vein were removed from the leaf sample, which were cut to less than 2mm. Then weighted the sample-spliced leaf 0.1g that were immersed 24h in the 25ml mixed liquid of 80% acetone and 95% ethanol in dark condition. The absorbance was measured at the bands of 440 nm, 645 nm and 663 nm with the UV-1800-vis spectrophotometerand and the chlorophyll content was determined by the Lichtenthaler method (Lichtenthaler et al., 1983).

Extracting the sensitive bands

The first derivative reflectance (FDR) was calculated using a first-difference transformation of the reflectance spectrum (Dawson and Curran, 1998; Moses and Andrew, 2006) as follows:

$$FDR_{\lambda(i)} = (R_{\lambda(j+1)} - R_{\lambda(j)}) / \Delta_{\lambda} \quad (1)$$

where, *FDR* is the first derivative reflectance at a wavelength *i*, midpoint between bands *j* and *j + 1*, $R_{\lambda(i)}$ is the reflectance of *j* band, $R_{\lambda(j+1)}$ is the reflectance of *j + 1* band, and Δ_{λ} is the difference in wavelengths between bands *j* and *j + 1*.

The coefficient of variation (*C.V*) is a statistics to weigh the variation degree in all data. *C.V* is calculated with the following equation (Håkanson, 2000):

$$C.V = (SD / MN) \times 100\% \quad (2)$$

where, *C.V* represents the coefficient variation of chlorophyll content under different planting densities. *SD* and *MN* are the standard deviation and average, respectively.

Data analysis

With the hyperspectral metadata in the present study, a new method of reduced precise sampling was designed by integrating the reduced sampling and precise sampling to realize fast analysis and feature extraction from the observed data. The NDVI, RVI (Pearson and Miller, 1972), DVI (Jordan, 1969) and PVI (Richardson and Wiegand, 1977) were constructed based on the sensitive spectral range extracted with the previous method and the analysis of characteristic spectrum. The best models were selected from the linear, exponential, logarithm and quadratic polynomial equations based the correlation relationship between the chlorophyll content and vegetation index. Multiple correlation coefficients (Abdi, 2007), F-test values of significance (Manjunath and Potdar, 2002), root mean square errors (RMSE) (Sadler et al., 2007) and standard error (SE) (Belia et al., 2005) were used as metrics for quantifying the amount of variation explained by the relationships developed and accuracy.

Cross-validation is widely used as a criterion to identify the best model (Wehrens and Linden, 1997). The main steps of this method are as follows: The original data is divided into *n* subsets and each subset is made as a test set, the rest subsets are training sets. So *n* models are made and the accuracy of these models is determined by the ways of determination coefficient, mean square error and standard error.

Results

Two plant types of winter wheat had the similar tendency of canopy spectral reflectance in different planting densities. The canopy spectra reflectance characteristics of PD 3 showed in Figure 1 (take joining stage as an example). There were two absorption bands in blue bands (center wavelength is 450 nm) and red bands (center wavelength is 650 nm) in canopy reflectance spectra of two winter wheat varieties, and reflectance decreased. However, there was an inconspicuous reflection peak in the 550 nm as the weak absorption to green light for chlorophyll. There was an absorption valley in red light (680 nm) which was Photosynthesis absorption valley. As the wheat leaf turned yellow, the absorption valley would decrease.

There was an obvious difference for the reflectance spectrum of two wheat varieties. The reflectance in visible spectrum (460-670 nm) was higher for “Jing9549” than “Le639”, while the reflectance was opposite in near infrared spectrum (810-1 100 nm).

Different plant types of winter wheat had the similar tendency of canopy spectral reflectance at joining stage. The canopy spectra reflectance characteristics were obtained from wheat (“Le639”) treated in different planting densities in Figure 2. It was very clear that there was a major difference in canopy reflectance spectra among different planting densities from the chart. As the density increased, reflectance decreased in the region of visible spectrum then canopy reflectance spectra zoom to near infrared spectrum from 780 nm, and reflectance increased with the density increased.

In some extent, *C.V* with canopy spectral reflectance of vegetation could show that changeable degree of time and space phase. The maximum *C.V* of canopy spectral reflectance in different planting densities appeared in the 670 nm, then 650, 600 and 500 nm, the minimum was 730 nm. *C.V* with canopy spectral reflectance of visible bands was more

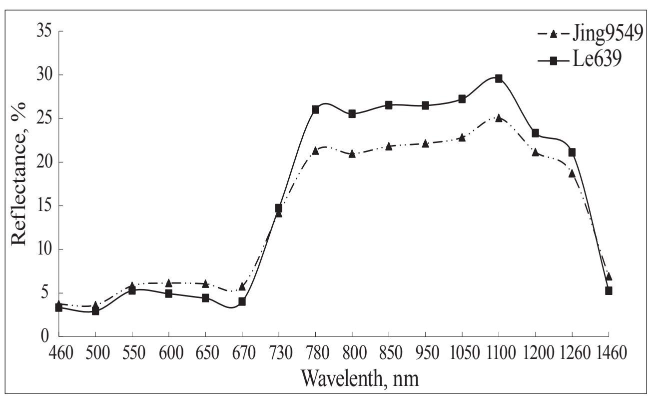


Fig. 1. The canopy spectra reflectance of different wheat cultivars at joining stage

than near infrared bands, except for 460, 550 and 730 nm. According to analysis, the spectral reflectance in 670 nm was most sensitive to the winter wheat agricultural parameters. In addition, it was similar with "Jing9549".

Figure 3 showed the first derivative canopy reflectance curve of different winter wheat varieties at joining stage. It revealed that the spectral red edge determined by LAI and chlorophyll content for wheat "Le639" was higher than wheat "Jing9549". The reason was that wheat Le639 had a higher LAI and chlorophyll content; finally, the red edge slope was larger for wheat "Le639".

The first derivative reflectance curve of canopy spectral for different wheat planting densities was different. As Figure 4 showed that, with planting densities increased, the reflectance increased in visual bands, while decreased in near infrared bands, and the obvious difference for first derivative reflectance of different wheat planting densities occurred at 730 nm band. As was known that the chlorophyll content and LAI increased quickly at the joining stage with the planting densities increased, so its canopy spectral red-edge increased and its position came close to near infrared band 730 nm. The result showed that the Red-edge position and the red edge slope were closely related to planting densities. Therefore, the band of 730 nm at the region of red-edge could be defined as sensitive band to study canopy spectra reflectance characteristics at joining stage.

Due to the impact of planting density to the population structure of winter wheat, it certainly caused the change of leaf canopy spectral information. The band of 730 nm was selected from the results as the sensitive band to describe the change low of leaf canopy spectral reflectance in winter wheat under different planting densities (take "Le639" for example) in Figure 5. From the figure, with advancing of the growth periods, reflectivity in 730 nm increased at first and then declined, and reached the top at booting stage. Reflectivity differed in different density treatment from stem elongation stage to booting stage, especially in PD3, PD1 and PD5, while the difference was smaller at other stages. Leaf canopy spectral reflectance in 730 nm of "Jing9549" changed as "Le639" along the growth periods, but the variation of reflectivity was smaller.

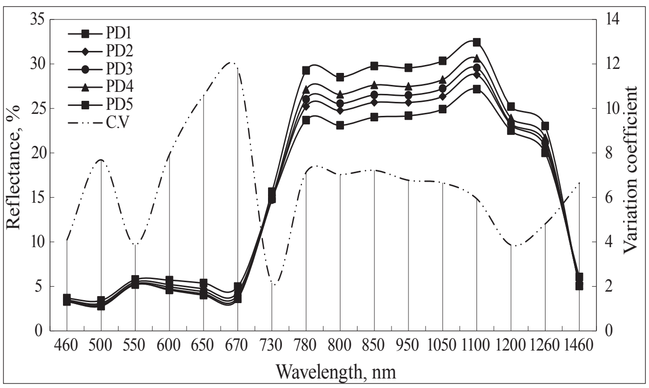


Fig. 2. The canopy spectra reflectance and C.V of winter wheat different planting densities (Le639)

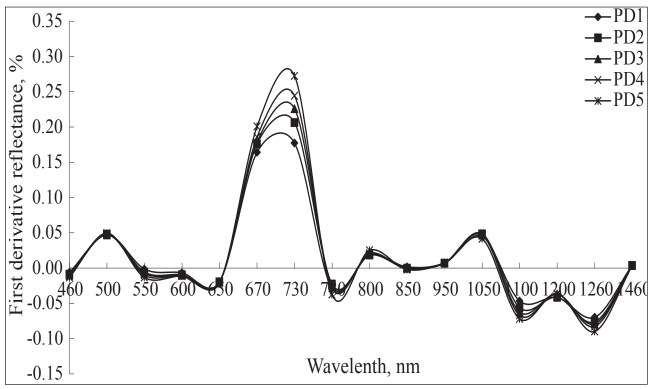


Fig. 4. The first derivative reflectance of wheat with planting densities (Le639)

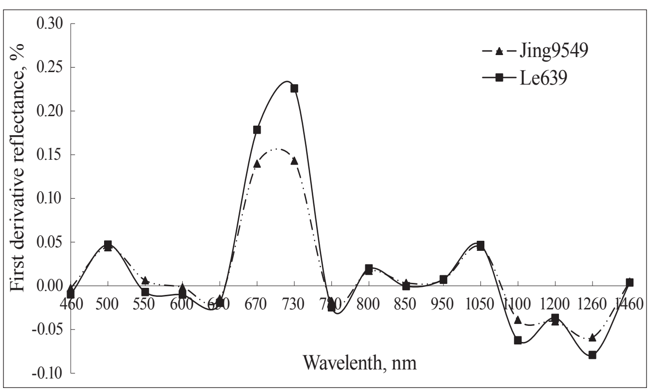


Fig. 3. The first derivative reflectance of different wheat cultivars

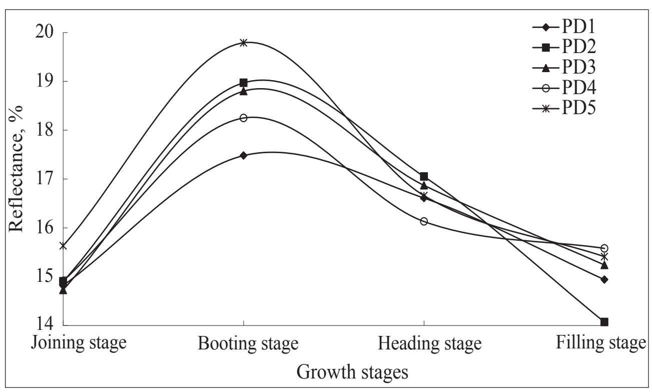


Fig. 5. Reflectance from Le639 canopy differential in density level at 730 nm at various growth stages

Correlation analysis between leaf chlorophyll content and reflectance of vegetation of different winter wheat cultivars was conducted in different growth periods. We found leaf chlorophyll content of winter wheat in different growth periods was negatively correlated with visible bands (460-670 nm), but positively correlated with near infrared bands (780-1 260 nm). In addition, there was the best correlation between reflectance of vegetation of "Le639" at booting stage and chlorophyll content in different winter wheat cultivars, while "Jing9549" was at jointing stage. In Figure 6, leaf chlorophyll content of "Le639" had a significant negative correlation with red band (650 nm) and max-relativity, then had a significant positive correlation above 730 nm and was very significantly correlated with near infrared bands between 780 nm and 1 260 nm, especially in 1200nm. While leaf chlorophyll content of "Jing9549" had a significant negative correlation with visible bands and had max-relativity in 670 nm, then had a significant positive correlation above 730 nm and with max-relativity between 1 200 nm and 1 260 nm. So the bands of 650, 670, 1 200 and 1 260 nm could be defined as sensitive bands to monitor leaf chlorophyll content of two different winter wheat cultivars.

Winter wheat population is various under different planting density, which will eventually affect the chlorophyll content of winter wheat. There was a good correlation between different planting densities and canopy spectral characteristic. So it was feasible to construct vegetation index with the visible band of 650, 670, 730 nm and near infrared band of 1 200, 1 260 nm which were selected from various bands. The vegetation indexes and chlorophyll content of winter wheat at key growth stage were analyzed, and the monitoring models of chlorophyll content were constructed. The formulation of linear, exponential, logarithm and quadratic polynomial equations were adopted to construct the models. The purpose of constructing the models is for prediction, so R^2 can be chosen as a measure of performance of the model (Boken and Shaykewich, 2002).

Table 1 showed that there was weak correlation between the chlorophyll content of "Jing9549" and the vegetation indexes, and it did not reach to significantly level at the booting stage and filling stage. However, the models at the jointing stage showed better test results. Compared with "Jing9549", the models at the growth stages of "Le639", R^2 obviously higher, especially at the booting stages, SE and RMSE were lower, all reached rather high level.

Mean variation tested with the cross-validation ranged from 0.0398 to 0.1101, and mean variation of Self-modeling was from 0.0179 to 0.0984, which was less than mean variation cross-validation. The study suggested that the canopy spectral characteristics could imitate and forecast the chlorophyll content of winter wheat. Overall, the models of two cultivars of winter wheat chlorophyll content showed better test results. Thus, spectral vegetation indices could be used to monitor the leaf chlorophyll content of different winter wheat cultivars planted with different densities at the key growth stages. The models based on the stage of jointing and booting for "Jing9549" and "Le639", respectively, would be best.

Previous research revealed better effects about composite models compared to single variable models (Jiang and Jardine, 2006). According to the character of the models, the composite monitoring models of chlorophyll content of different cultivars were constructed at the booting stage and jointing stage.

In the Table 2, the F values of composite models were higher than the values of F-crit, which indicated that two models reached to great significant level at 0.01. Compared with single variable models, the composite models had higher R^2, and lower SE, RMSE. It suggested that the composite models were better than single variable models.

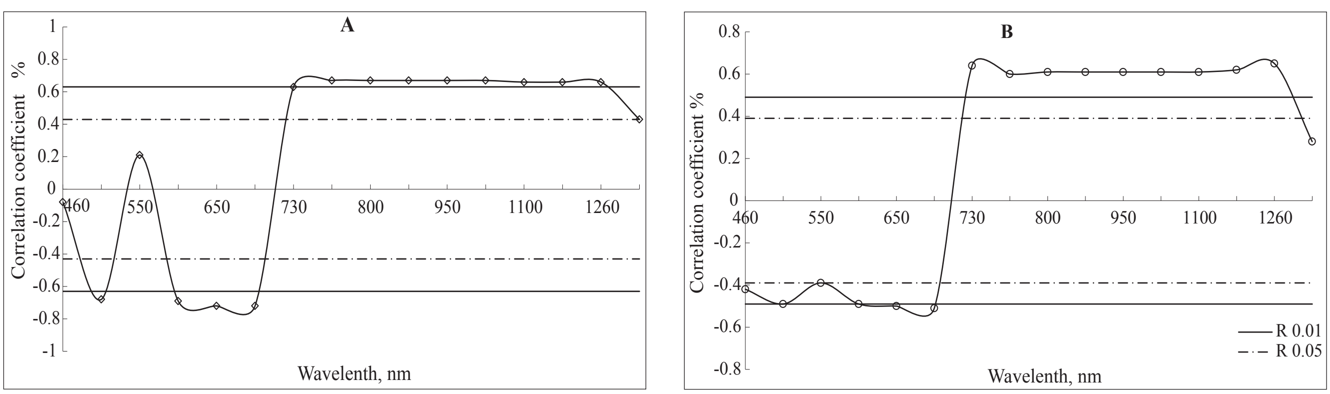


Fig. 6. Correlation between leaf Chlorophyll content and canopy spectra reflectance at various bands (A: Le639; B: Jing9549)

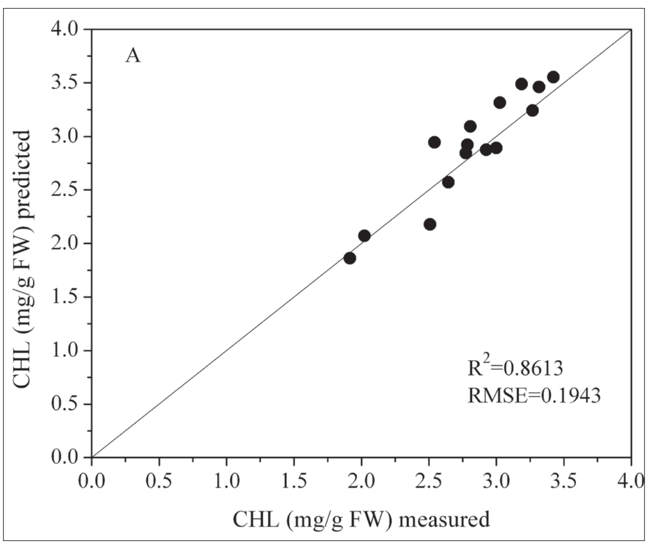
In order to test whether these regression models were reliable and applicable for LNA estimation, the independent data set from Exp. 2 were used to test the performance of the two models. The CHL (mg/g FW) was predicted from PVI (1200, 730) and DVI (1260, 730), resulting in a prediction of CHL [R^2 = 0.8613, RMSE = 0.1943, slope = 1.1001 ($p < 0.01$), intercept = -0.2316] for "Jing9549" at joining stage (Figure 7A). The band combination of DVI (1200, 650) and DVI (1200, 730) was an excellent predictor of CHL [R^2 = 0.8871, RMSE = 0.0922, slope = 0.8266 ($p < 0.01$), intercept = 0.5685] for "Le639" at the booting stage (Figure 7B).

Table 1
The best monitoring models of leaf Chlorophyll content and results of test

Stage	Variation	Band combination	Model	R^2	SE	RMSE	Test methods: Average deviation of model itself	Test methods: Average deviation of cross test
Joining stage	Jing9549	1200, 730	y=0.6876×exp(-0.1027×PVI)	0.4269	0.3793	0.1488	0.0984	0.1101
		1260, 730	$y=0.0047\times DVI^2+0.0484\times DVI+2.2936$	0.4124	0.3753	0.1457	0.1000	0.1172
	Le639	1200, 670	$y=-0.4266\times NDVI^2+4.0642\times NDVI-0.2572$	0.5283	0.2727	0.0769	0.0858	0.0939
Booting stage	Le639	1200, 650	y=3.145×Ln(DVI)-6.8817	0.6562	0.1641	0.1703	0.0402	0.0532
		1200, 730	$y=-0.0279\times DVI^2+0.7247\times DVI-0.9890$	0.6973	0.1541	0.1598	0.0392	0.0521
Heading stage	Jing9549	1260, 730	$y=0.0666\times DVI^2-1.7545\times DVI+16.6080$	0.3722	0.1706	0.0317	0.0220	0.0308
	Le639	1200, 670	y=-6.2103×Ln(DVI)+24.8660	0.5388	0.1601	0.0288	0.0241	0.0315
Filling stage	Le639	1260, 730	y=3.1626×exp(0.0569×DVI)	0.6206	0.1643	0.0659	0.0179	0.0398

Table 2
Combined models of VI both in joining and booting stages and leaf chlorophyll content of different winter wheat

Stage	Variation	Model	R^2	F-test	F-crit	SE	RMSE
Joining stage	Jing9549	y=0.0214×exp(-0.4839×PVI(1200,730))/DVI(1260,730)	0.5703	10.1688	6.93	0.3354	0.1358
Booting stage	Le639	y=0.1895×DVI(1200,650)+0.5305×DVI(1200,730)-0.0154×DVI(1200,650)×DVI(1200,730)-2.7710	0.7165	8.4229	5.95	0.1491	0.0239



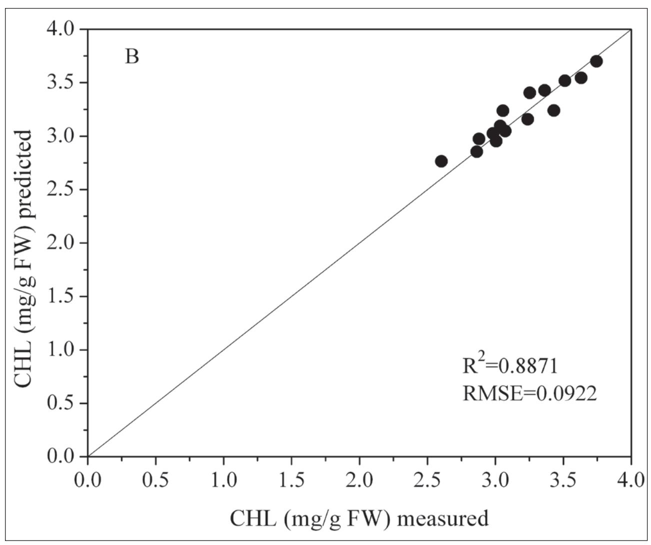


Fig. 7. Predictions of CHL using composite models. (A) Jing9549, Joining stage and (B) Le639, Booting stage. Both are shown in 1:1 line

Discussion

There is a close relation between planting density and crop population structure, so the change of population structure will inevitably affect canopy spectral information. It has practical meaning for the research on high-yielding, high-efficiency and high-quality cultivation of winter wheat, though using sensitive bands to build models to predict winter wheat chlorophyll content. Heretofore, according to our knowledge, relatively less research has been carried out on winter wheat canopy spectral information in different planting density. In this paper, the results showed that there were significant differences of winter wheat canopy spectral in different planting density. As the density increased, the reflectance in the visible region reduced and increases in near infrared region. This result was consistent with the research result of different nitrogen application on winter wheat canopy reflectance spectra (Feng et al., 2008).

In the established models, the composite model can contain the explanatory power of the variables to avoid the problem of omitted variable bias. Therefore, the test results of composite models are superior to single variable models. In addition, it was more accurate and more reliable to predict winter wheat chlorophyll content.

There are many influence factors on canopy spectral reflectance whose characteristic is made up of the characteristic of crops spectral reflectance and background soil (Xue et al., 2004), such as the incident angle of solar beam, bidirectional reflectance, aerosol and wind speed so on external factors during the spectrometry. While canopy spectral reflectance also can be influenced by fertilizer amount, type, method and the condition of the crop growth environment (Zhu et al., 2002). However, this study involved only two factors, planting density and two winter wheat cultivars, so it is necessary to study more deeply in improving monitoring precision of chlorophyll content.

Conclusions

The research results showed that *C.V* and first derivative reflectance of canopy spectral bands could well determine spectral sensitive bands of winter wheat. The determination of sensitive bands was beneficial to decrease information redundancy and improve data treatment efficiency.

The plant type of winter wheat would result in the situation that there was an obvious difference in near infrared bands for the canopy spectral of different wheat varieties. “Le639” whose LAI and chlorophyll content were higher than “Jing9549” is a high-stem and ownless type. Therefore, the reflectance in near infrared bands was increased and the “red shift” happened for “Le639”.

There was a significant relationship between four selected vegetation indexes and chlorophyll content. It showed that chlorophyll content could be well simulated and predicted based on the canopy spectral at the key development stage of winter wheat. In this article, the key development stages were at jointing stage and booting stage for “Jing9549” and “Le639”, respectively, through the cross validation.

Acknowledgments

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