

Analysis of Changes in Agronomic Parameters and Disease Index of Rapeseed Leaf Leukoplakia Based on Spectra

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Abstract. The non-destructive, rapid and accurate monitoring diagnosis of rapeseed diseases is of significance for sustainable development of rapeseed production and environment protection. The spectrum data of rapeseed leaf leukoplakia were collected in the experimental Farm of Jiangsu Academy of Agricultural Science in 2013 and 2014. Firstly, the common distinctive bands of the disease and the health were found by comparing reflectance spectrum of leaves in the field and under black background. The results showed that with the progress of the growth period, reflectance of disease leaves decreased earlier than healthy leaves. It was the best period to identify rapeseed leukoplakia from 11 days after early anthesis to 9 days after finish flowering to identify rapeseed leukoplakia in the field due to during this period the reflectance of healthy leaves remained at 35 % while the disease diseased to 30 %. The sensitive band was in the range of 760–1080 nm. The correlation among disease index (DI), agronomic parameters, and the reflectance of the disease samples were analysed, and the results showed that there were high correlations between DI, and agronomic parameters and reflectance, e.g., the correlation between the leaf moisture content and the reflectance in 460 nm, 550 nm, 650 nm, 710 nm, 760 nm, 1480 nm, and 1600 nm, between the leaf nitrogen content and the reflectance in 810 nm, 870 nm, 1080 nm, 1280 nm, 1320 nm, 1540 nm, 1600 nm, 1650 nm, and 1700 nm, and between the SPAD value and the reflectance in 1200 nm, 1280 nm, and 1540 nm had significance with $p < 0.01$. The quantitative models of agronomic parameters based on reflectance were developed by stepwise regression, principal component analysis, and curve fitting. The data of rapeseed leukoplakia in 2013 and rapeseed virus in 2014 were used to test. The results showed that in the same disease test, the quantitative models of moisture content based on reflectance were fit well. In the different disease test, the quantitative models were fit badly except the model of moisture content. The model on moisture content performed reasonably well, though performance of precision could probably be improved by further analysis, and the paper would provide a basis for spectrum-based identifying of rapeseed leaf leukoplakia.

Keywords: Rapeseed leukoplakia · Spectrum reflectance · Disease index · Agronomic parameters

1 Introduction

Rapeseed is one of very important oilseed crops in the world, and its plant area in normal year is about 18–30 million ha. The plant area of rapeseed in China is about 6–7 million ha, and its total yield is about 10–13 million tons, which ranks the fifth place in crop production in China [1]. However, the control for diseases and insect pests is the key to crop production with high yielding, good quality, better benefit, ecology, and safety, and the traditional control for diseases and insect pests depends on manned or machined application of pesticide, which has some obvious disadvantages such as bigger work intensity, high cost, and environmental problems, etc. [2–5].

The spectrum technology is a new subject that detects changes in properties and shapes of object as non-touch manner through using physics theories, mathematics methods, and geography rules [6]. In recent years, studies on crop spectrum technology have made rapid progress. Some researchers pointed out that vegetation reflective spectrum can denote its health status, and some relevant physiological biochemistry indices, e.g. Xue *et al.* [7, 8], Zhang *et al.* [9], Li *et al.* [10], and Hou *et al.* [11] developed the reflective spectrum-based models of plant nitrogen content for rice, wheat, cucumber, orange, and cotton, respectively; Tian *et al.* [12, 13], Zhang *et al.* [14], Mao *et al.* [15], and Fu *et al.* [16] proposed the reflective spectrum-based quantitative models of plant water content for rice, wheat, rapeseed, lettuce, and alfalfa, respectively; and Xie *et al.* [17], Zheng *et al.* [18], Chen *et al.* [19], Zou *et al.* [20], and Li *et al.* [21] set up the reflective spectrum-based quantitative models of leaf SPAD value for rice, wheat, corn, tea plant, and apple. However, few studies have focused on the spectrum-based quantitative models for agronomic parameters of crop disease sample.

The objectives of this research were to find characteristic spectrum band using comparison of the reflective spectrum of health and disease leaf for rapeseed, to reveal the quantitative relationships between the characteristic spectrum and agronomic parameters of disease leaf for rapeseed, and to provide a basis for spectrum-based identifying of rapeseed leaf leukoplakia.

2 Materials and Methods

We conducted field experiments at the Jiangsu Academy of Agricultural Sciences farm, Nanjing, China (32°03'N) during the 2012 to 2013, and 2013 to 2014 growing seasons. The soil type of the experimental area is a hydric anthrosol. Soil test results indicated the following: organic carbon, 13.8 g kg⁻¹; whole nitrogen, 2.03 g kg⁻¹; available phosphorus, 20.30 mg kg⁻¹; available potassium, 139.00 mg kg⁻¹; and pH, 7.31.

2.1 Materials

This study used 3 rapeseed cultivars bred by Institute of Economical Crops of Jiangsu Academy of Agricultural Sciences: (V1) Ningyou18 (normal), (V2) Ningyou16 (normal), and (V3) Ningza19 (hybrid).

2.2 Methods

2.2.1 Experimental Conditions and Design

The experiments on cultivars were a randomized complete design with 3 varieties (listed above) and 3 replications. The sowing date was Oct. 8 and the transplanting date was Nov. 9 for 2012 to 2013, and Oct. 3 and Nov. 1 for 2013 to 2014, respectively. The 5-leaf seedlings (30, and 33 seedling days for 2012 to 2013, and 2013 to 2014) were transplanted into 60- by 30-cm spacings in 7.98- by 3.50-m subplots. The fertilizer included 90 kg N ha⁻¹ (as urea), 90 kg P₂O₅ ha⁻¹, and 90 kg K₂O ha⁻¹. The basal: overwinter: elongation fertilizer ratio was 5:3:2. The 15 kg borax ha⁻¹ was sprayed on foliage after elongation for 2013 to 2014. Other management activities followed local production practices.

2.2.2 Measurements

Spectrum: we used CropScan MS16R Multispectral Radiometer with 16 wave bands (460, 550, 650, 710, 760, 810, 870, 1080, 1200, 1280, 1320, 1480, 1540, 1600, 1650, and 1700 nm) to collect reflective spectrum of healthy and leukoplakia disease leaf at 31.1° view field angle under field canopy background and under black cloth.

- (1) Reflectance spectrum of leaves in the field: we randomly selected 5 to 10 healthy, and leukoplakia disease leaf, and investigated their reflective spectrum during 10:00 to 12:00 AM under clearness or few cloud every 7 days for Mar. 15 to May 3 in 2013, and for Apr. 9 to May 6 in 2014. At measuring, the spectrum sensor should be plumbed with ground, and hold out about 30 cm high over target leaf. The data of each leaf were measured in 5 repeats, and their average values were computed.
- (2) Reflectance spectrum of leaves under black background: after finishing measurement in the field, the target leaves were taken from the plant, and lain on the black background to examine their reflective spectrum taking nature light as light source with the same method in (1).

Leaf water content: leaf fresh weights were weighed before reflectance spectrum of leaves was determined under black background, and after finishing measurement under black background, put in oven, and dried in 30 min. at 105°C, then at 80°C until reaching a stable weight, where dry weight were weighed using a 0.001 g electro-level. Leaf water content can be computed.

Leaf SPAD value: we used SPAD-502 Chlorophyll meter to measure SPAD value of upside, middle part, and basal for the healthy and the leukoplakia disease leaves, and their average values were computed.

Disease index (DI): we acquired leukoplakia disease leaves images using Nikon P5000 numeral camera under black background and 1 cm² reference, gained

pixels and numbers of leukoplakia disease spot, and leaf area in Photoshop CS4.0, and the DI was computed.

All experiment data were analysed using Microsoft EXCEL.2003 and SPSS ver. 19.0.

2.2.3 Model Validation

The models developed in this paper were validated by calculating the correlation (R), the average absolute difference (d_a), the ratio of d_a to the average observation (d_{ap}), the standard error of absolute difference (S_d , like the root mean squared errors, RMSE (Evers *et al.* 2005)) [22, 23], and 1:1 figure of simulated and observed properties, which were defined as:

$$d_i = X_{Oi} - X_{Si}$$

$$d_a = \frac{1}{n} \sum_{i=1}^n d_i$$

$$\bar{X}_O = \frac{1}{n} \sum_{i=1}^n X_{Oi}$$

$$d_{ap}(\%) = d_a / \bar{X}_O \times 100$$

$$S_d = \sqrt{\frac{\sum_{i=1}^n (d_i - d_a)^2}{n - 1}}$$

with i = sample number, n = total number of measurements, $n - 1 = n$ when $n \geq 30$, X_{Si} = simulated value, and X_{Oi} = observed value.

3 Results

3.1 Changes in Reflectance Spectrum of Healthy and Leukoplakia Disease Leaves in Different Growth Periods for Rapeseed

3.1.1 Reflectance Spectrum of Leaf in Field

The field experiment data in 2013 showed that the changes in spectrum reflectance of rapeseed leaf in field were decreasing trend with growth period duration, and the biggest spectrum reflectance (around 810 nm) from early anthesis to the 17th day after end anthesis was reduced from 45 % to 30 %, in that the decline for spectrum reflectance of leukoplakia disease leaves was earlier than that of the healthy; The biggest spectrum reflectance from the 9th day after early anthesis to the 9th day after end anthesis was kept at around 35 % for healthy leaves, and reduced at around 30 % for leukoplakia disease leaves. Thus, this period was the optimum time for identifying rapeseed leukoplakia disease in field based on reflectance spectrum, and the average spectrum reflectance was about range of 30 % to 35 % for leukoplakia disease leaves; In total, the spectrum reflectance in near infrared band (760–1080 nm) for healthy

leaves was higher than that of the leukoplakia disease, while there was a reverse status in short-wave infrared band (1080–1700 nm) except for around 1500 nm moisture absorb peak, and there was no obvious difference in others; In addition, because of no obvious difference for color of leukoplakia disease and healthy leaves in field, there was no obvious difference for the spectrum reflectance in visible light band (460–760 nm) for leukoplakia disease and healthy leaves (shown in Figs. 1, 2, 3 and 4).

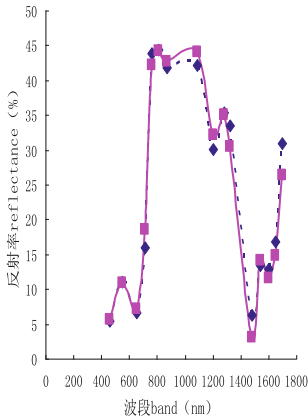


Fig. 1. Reflectance spectrum of *Brassica napus* leaves under the background of field groups in early anthesis (4.2)

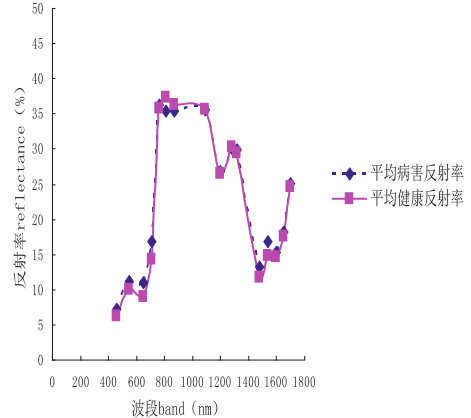


Fig. 2. Reflectance spectrum of *Brassica napus* leaves under the background of field groups 11 days after anthesis (4.12)

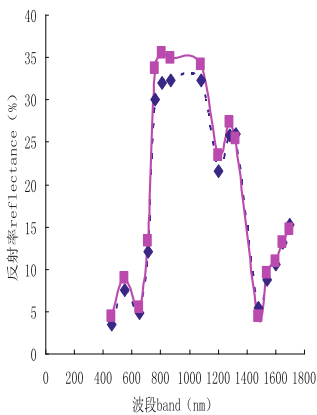


Fig. 3. Reflectance spectrum of *Brassica napus* leaves under the background of field groups 9 days after finish flowering (4.25)

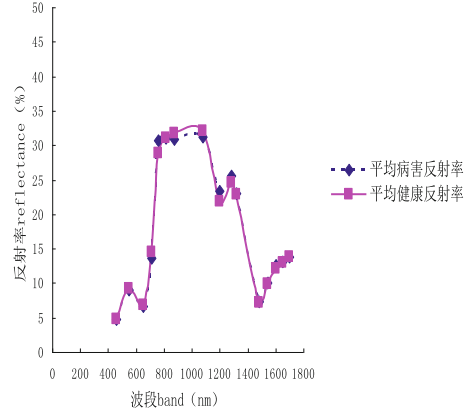


Fig. 4. Reflectance spectrum of *Brassica napus* leaves under the background of field groups 17 days after finish flowering (5.3)

3.1.2 Reflectance Spectrum of Leaf in Black Background

In order to eliminate the effects of soil environmental factors in field on reflectance spectrum of leaf, Observations of reflectance spectrum for leaf at various growth period were made under black background (shown in Figs. 5, 6 and 7), the results showed that the spectrum reflectance in visible light band (460–760 nm) for healthy leaves less than that of the leukoplakia disease, and the reasons of that were that chlorophyll was destroyed when the leukoplakia disease occurred, absorption to light from coloring matter was reduced, and this made the spectrum reflectance in visible light band increase; In near infrared band (760–1080 nm), because the rapeseed leaf cell structure was broken when the leukoplakia disease occurred, the number of cell layer was diminished, this made the light transmission increase, and the spectrum reflectance was weakened correspondingly; Although the spectrum reflectance in near infrared band for healthy leaves was slight decreasing with rapeseed growth period duration, its highest value was from 70 % to around 65 %, there was more obvious diminishing for that of the leukoplakia disease leaves, and in total, the distance of spectrum reflectance between the healthy and the leukoplakia disease leaves was gradually increased in advantages to leukoplakia disease identifying.

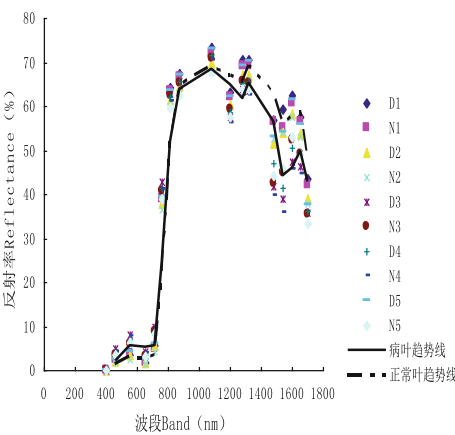


Fig. 5. Leaf reflectance under natural light in early anthesis (4.2)

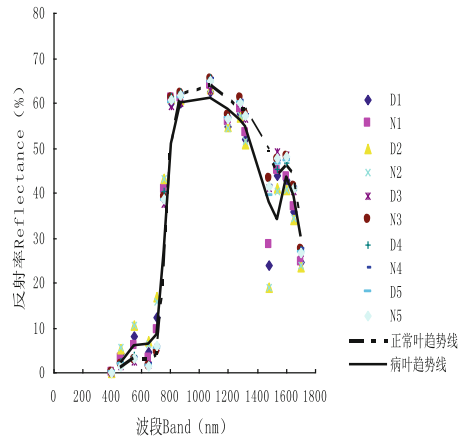


Fig. 6. Leaf reflectance under natural light 1 day after finish flowering (4.17)

Note: D1, D2, D3, D4, and D5 denote the disease leaf reflectance, and N1, N2, N3, N4, and N5 denote the normal leaf reflectance, respectively.

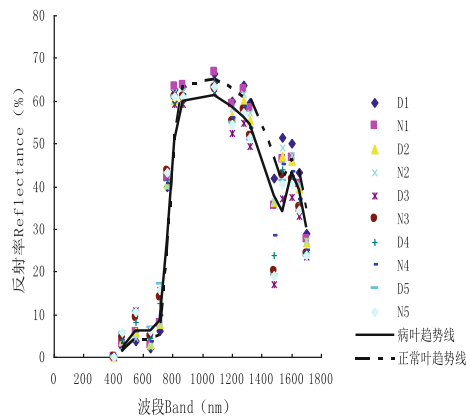


Fig. 7. Leaf reflectance under natural light 9 days after finish flowering (4.25)

3.2 Correlation Between the Reflectance and the Disease Index (DI), and Agronomic Parameter

3.2.1 Correlation Between the Reflectance and the DI, and Agronomic Parameter

The correlation between the reflectance and the disease index (DI), and agronomic parameter for disease leaves was shown in Table 1. We saw that the correlation between the reflectance and the DI at 1200 nm, 1480 nm, and 1600 nm was significant with $p < 0.05$, that of between the reflectance and the disease leaf moisture content in short-wave infrared band 1320 nm to 1650 nm was significant with $p < 0.01$, in which there was the highest negative correlation, -0.810 , at 1540 nm, that of between the reflectance and the disease leaf nitrogen content in band 810 nm to 1320 nm, and 1540 nm to 1700 nm was significant with $p < 0.01$, in which there was correlation larger than 0.70 at band 810 nm, 870 nm, and 1080 nm, and that of between the reflectance and the disease leaf chlorophyll SPAD value in band 1200 nm, 1280 nm, and 1540 nm was significant with $p < 0.01$.

Table 1. Correlation coefficients between reflectance and DI, agronomic parameters (n = 22)

Band	Disease index (DI)	Moisture content	Nitrogen content	SPAD value
460	-0.173	0.471*	0.140	-0.063
550	-0.216	0.471*	0.171	-0.173
650	-0.113	0.299	0.153	-0.362
710	-0.191	0.449*	0.204	-0.171
760	-0.293	0.398	0.034	-0.030
810	0.344	0.199	-0.743**	-0.195
870	0.123	0.203	-0.730**	-0.191
1080	-0.018	0.170	-0.719**	-0.230
1200	0.470*	-0.465*	-0.593**	-0.640**

(continued)

Table 1. (continued)

Band	Disease index (DI)	Moisture content	Nitrogen content	SPAD value
1280	0.279	−0.471*	−0.660**	−0.593**
1320	0.316	−0.657**	−0.566**	−0.506*
1480	0.512*	−0.732**	−0.339	−0.496*
1540	0.198	−0.810**	−0.679**	−0.555**
1600	0.425*	−0.777**	−0.556**	−0.462*
1650	0.303	−0.750**	−0.657**	0.116
1700	0.014	−0.400	−0.670**	−0.339

Note: The asterisk * denote significant correlation at $p < 0.05$, and the asterisk ** denote significant correlation at $p < 0.01$; $r_{(20, 0.05)} = 0.423$, and $r_{(20, 0.01)} = 0.537$.

3.2.2 Changes in Agronomic Parameters with the DI

The field experiment data in 2013 showed that there were declines in the moisture content, the nitrogen content, and the SPAD value for rapeseed disease leaf (shown in Figs. 8, 9 and 10) with the DI increase, which revealed that some leaf physiological indices were in response to the effects of leukoplakia disease on rapeseed leaf, of that there was better correlation between the SPAD value and DI. However, we known that the SPAD value for rapeseed disease leaf increased with its nitrogen content increase through correlation analysis (Fig. 11), and the studies in literature [24–26] also indicated that there was higher correlation between the SPAD value and nitrogen content. Therefore, we considered that the SPAD value can be as one of index indicated severity degree of leukoplakia disease and leaf nitrogen content for rapeseed.

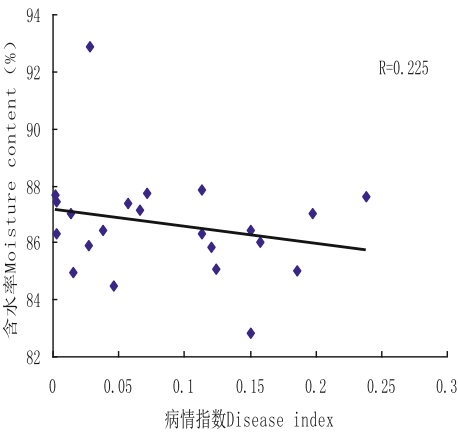


Fig. 8. Relationship between moisture content and DI

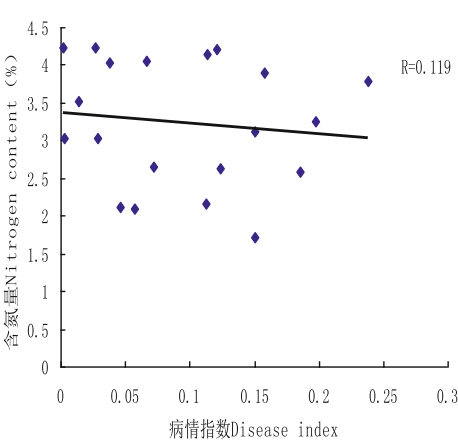


Fig. 9. Relationship between nitrogen content and DI

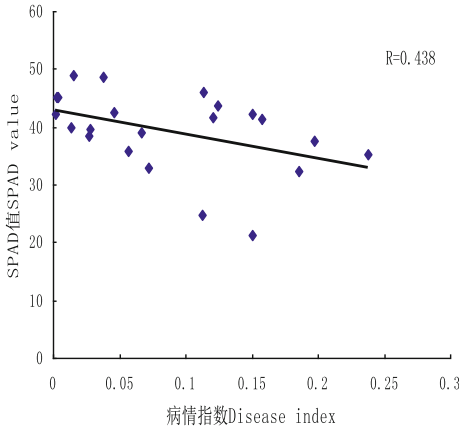


Fig. 10. Relationship between SPAD value and DI

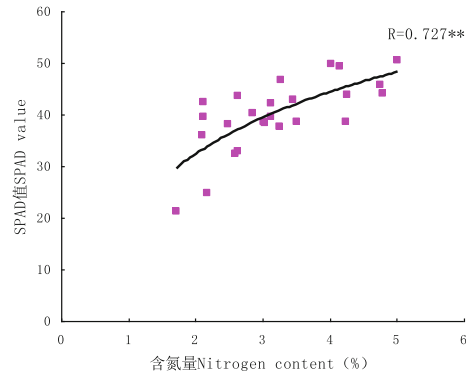


Fig. 11. Relationship between nitrogen content and SPAD value

3.3 Spectrum-Based Quantitative Analysis of Agronomic Parameters

3.3.1 Reflectance-Based Quantitative Analysis of Agronomic Parameters

Stepwise Regression. In order to reflectance-based quantify some leaf agronomic parameters, it need to develop the regression models between the multi-spectrum variables and leaf agronomic parameters, such as the moisture content, the nitrogen content, and the SPAD value of disease leaf. The correlation analysis indicated that there was higher correlation of reflectance for various bands, and a serious issue with multicollinearity (Table 2). Thus, we found regression functions between the agronomic parameters and the reflectance using multiple stepwise regression and principal component analysis, of that the quantitative model for the disease leaf moisture content was taking the reflectance at 1540 nm (Eq. 1 in Table 3), for the disease leaf nitrogen content taking the reflectance at 810 nm (Eq. 2 in Table 3), and for the disease leaf nitrogen content taking the reflectance at 1280 nm, 1080 nm, and 650 nm as independent variable (Eq. 3 in Table 3), respectively, and the variable inflate factor (VIF) of these regression functions were less than 10, which revealed that there were higher tolerance among reflectance in various bands. The statistics test for the models was shown in Table 4.

Curve Fitting. Some studies in literature [27, 28] showed that there was larger difference when various models were fitted using same independent variable, thus, we fitted the reflectance-based equation on moisture content, nitrogen content for disease leaf chosen linearity ($y = ax + b$), logarithm ($y = \log_a bx$), quadratic ($y = ax^2 + bx + c$), and exponential equation ($y = a^{bx}$), and the decision coefficient was shown in Table 5. Of that the decision coefficients (R^2) of the reflectance-based quadratic

Table 2. Correlation coefficients between the reflectance for various bands

	460	550	650	710	760	810	870	1080	1200	1280	1320	1480	1540	1600	1650	1700
460	1.00															
550	0.96	1.00														
650	0.93	0.98	1.00													
710	0.86	0.95	0.97	1.00												
760	0.8	0.87	0.81	0.84	1.00											
810	-0.41	-0.41	-0.42	-0.42	-0.12	1.00										
870	-0.47	-0.42	-0.44	-0.40	-0.09	0.92	1.00									
1080	-0.45	-0.38	-0.40	-0.34	-0.05	0.82	0.98	1.00								
1200	-0.69	-0.78	-0.75	-0.79	-0.65	0.79	0.67	0.56	1.00							
1280	-0.73	-0.76	-0.76	-0.76	-0.54	0.85	0.86	0.81	0.93	1.00						
1320	-0.72	-0.74	-0.75	-0.75	-0.52	0.80	0.87	0.84	0.87	0.99	1.00					
1480	-0.76	-0.85	-0.84	-0.89	-0.73	0.71	0.66	0.58	0.96	0.94	0.92	1.00				
1540	-0.72	-0.81	-0.75	-0.79	-0.81	0.57	0.40	0.28	0.93	0.77	0.70	0.89	1.00			
1600	-0.75	-0.83	-0.79	-0.82	-0.74	0.71	0.63	0.55	0.98	0.92	0.89	0.97	0.94	1.00		
1650	-0.73	-0.79	-0.77	-0.79	-0.63	0.78	0.78	0.72	0.95	0.98	0.97	0.97	0.84	0.97	1.00	
1700	-0.58	-0.57	-0.59	-0.55	-0.31	0.78	0.91	0.92	0.71	0.91	0.94	0.76	0.49	0.72	0.87	1.00

Table 3. Stepwise regression of reflectance and parameters (n = 20)

Parameter	Model	F	Sig.	R ²	VIF	Eq
Moisture content	$Y = 112.7 - 0.6R_{1540}$	36.334	0.000	0.657	1.000	1
Nitrogen content	$Y = 20.805 - 0.28R_{810}$	22.119	0.000	0.551	1.000	2
SPAD	$Y = -143.658 - 4.475R_{1280} + 6.849R_{1080} - 2.302R_{650}$	15.914	0.000	0.737	3.688	3

Note: R_{650} , R_{810} , R_{1080} , R_{1280} , and R_{1540} denote the disease leaf reflectance at 650 nm, 810 nm, 1080 nm, 1280 nm, and 1540 nm, respectively.

Table 4. Coefficient of Equations and t-test

Parameter	Unstandardized coefficients	Corresponding variable	t	Sig.
Moisture content	0.005	R_{760}	4.298	0.000
	0.638	constant	11.972	0.000
Nitrogen content	-0.28	R_{810}	-4.703	0.000
	20.805	constant	5.563	0.000
SPAD	-4.475	R_{1280}	-6.261	0.000
	6.849	R_{1080}	4.524	0.000
	2.302	R_{650}	-3.520	0.003
	-143.685	constant	-1.975	0.065

equation on moisture content, and the exponential equation on nitrogen content were the largest, and the t-test on parameters in these two equations was shown in Table 6. We saw that all parameters in the quadratic equation on moisture content, $Y = 2.251 - 0.071R_{760} + 0.001R_{760}^2$, had significant with $p < 0.05$, and the parameter a in the exponential equation on nitrogen content had no significant in the t-test.

Table 5. Curve estimation of moisture content, nitrogen content and chosen bands

Parameter	Independent variable	Models	R ²	F	Sig.
Moisture content	R ₇₆₀	linearity	0.480	18.472	0.000
		logarithm	0.460	17.069	0.001
		quadratic	0.620	15.487	0.000
		exponential	0.476	18.172	0.000
Nitrogen content	R ₈₁₀	linearity	0.551	22.119	0.000
		logarithm	0.550	21.998	0.000
		quadratic	0.553	10.498	0.001
		exponential	0.571	23.974	0.000

Table 6. Coefficient of equations and t-test

Equation	Unstandardized coefficients	Parameter	t	Sig.
Moisture content	-0.071	b	-2.451	0.024
	0.001	a	2.642	0.016
	2.251	c	3.675	0.002
Nitrogen content	-0.096	b	-4.896	0.000
	1282.838	a	0.813	0.427

Principal Components Analysis. We selected the higher correlation bands with moisture content (460 nm, 550 nm, 650 nm, 710 nm, 760 nm, 1480 nm, and 1600 nm), nitrogen content (810 nm, 870 nm, 1080 nm, 1280 nm, 1540 nm, 1650 nm, and 1700 nm), and the SPAD value (1200 nm, 1280 nm, 1320 nm, and 1540 nm) for the disease leaf, made dimension reduction, and found principal components. Then, regression analysis was made selecting the first principal components as independent variable.

Because almost the parameters in regression equation with the highest decision coefficient had no significant in t-test (Table 7), and the decision coefficients were less than that of the stepwise regression, it was seen that principal components regression was not suitable for regression of multi-band combination and agronomic parameters.

Table 7. Coefficient of principal components regression equation and t-test

Model	Unstandardized coefficients	Corresponding variable	t	Sig.
Moisture content	-0.001	c	-1.768	0.095
	0.005	b	-1.233	0.234
	0.007	a	-0.665	0.515
	0.861	d	90.769	0.000
Nitrogen content	-0.734	c	-2.342	0.032
	-0.093	b	-0.716	0.485
	0.069	a	0.531	0.603
	3.353	d	17.752	0.000
SPAD	-2.866	c	-0.535	0.600
	-6.698	b	-1.874	0.078

(continued)

Table 7. (continued)

Model	Unstandardized coefficients	Corresponding variable	t	Sig.
	−3.619	a	−1.139	0.271
	31.400	d	9.906	0.000

3.3.2 Model Validation

The stepwise regression models developed in this paper were validated by using independent experiment data in 2013, and 2014, and the results showed that the average absolute difference (da), the ratio of da to the average observation (dap) of simulation and observation in moisture content were less than 5 %, and that of the nitrogen content, and the SPAD value were larger than 20 % except for da for the nitrogen content (Tables 8 and 9). The 1:1 figure on simulation and observation in the moisture content, the nitrogen content, and the SPAD value are represented in Figs. 12, 13, 14, 15, and 16. As illustrated by comparisons of 1:1 map, the model on moisture content performed reasonably well, though performance of precision could probably be improved by further analysis.

Table 8. Stepwise regression model test of the same disease samples

Parameters	N	d _a	d _{ap} (%)	r	t	Sig.
SPAD	5	22.684	52.901	−0.512	7.247	0.002
moisture content	10	3.477 %	4.138	0.810	−1.137	0.269
nitrogen content	10	3.424 %	85.465	0.748	0.520	0.616

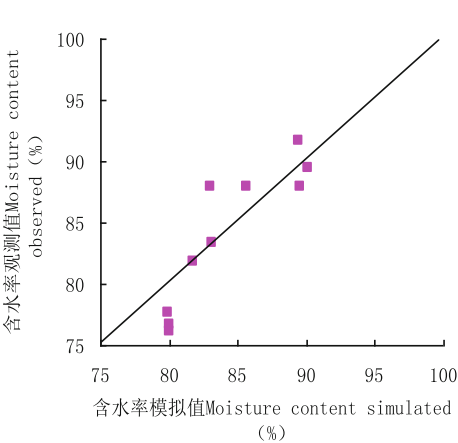


Fig. 12. Comparison of observation and simulation in moisture content of the same disease

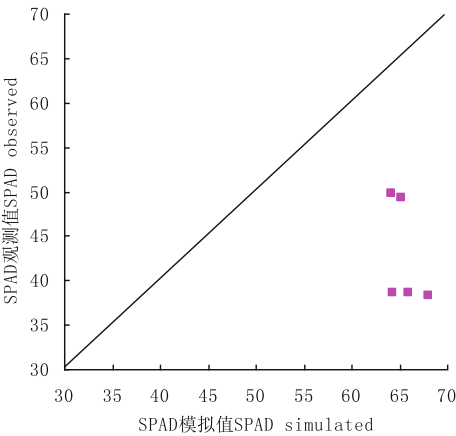


Fig. 13. Comparison of observation and simulation in SPAD value of the same disease

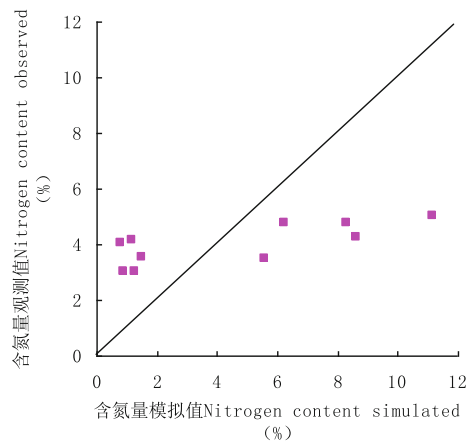


Fig. 14. Comparison of observation and simulation in nitrogen content of the same disease

Table 9. Stepwise regression model test of different diseases samples

Parameters	N	d _a	d _{ap} (%)	r	t	Sig.
SPAD	21	20.260	76.849.	0.775	-12.934	0.000
moisture content	21	5.268 %	6.083	0.537	0.281	0.781

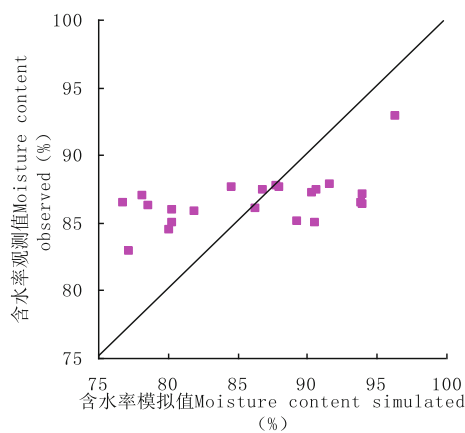


Fig. 15. Comparison of observation and simulation in moisture content of different diseases

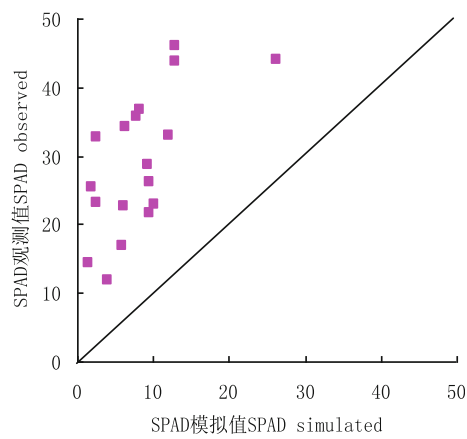


Fig. 16. Comparison of observation and simulation in SPAD value of different diseases

4 Discussion

4.1 The Difference of Reflectance Spectrum Between the Normal and Disease Leaf Under Field Nature Condition Was Reduced

Because there was large difference of the severity degree of disease leaves collected in various growth periods, of that the severity degree of partial disease leaves was less, whose leaf color had no difference for the normal and disease leaves basically, and its chlorophyll structure was not destroyed, the correlation between the reflectance in visible bands (determined by chlorophyll absorption) and the DI was not high, and there was a significant correlation only in near infrared band (1480 nm), which indicated that there were large effects of leaf moisture content on disease leaf reflectance. This was in accordance with the low correlation between the reflectance in visible bands and the SPAD value.

4.2 There Were Serious Multicollinearity Among Bands

Because there was serious multicollinearity among bands, the independent variable with higher correlation was excluded using the stepwise regression, the multiple linear regression equations between the DI, the moisture content, the nitrogen content, and the SPAD value and reflectance were set up.

4.3 The Effects of Leaf Thickness on SPAD Value Were in Accordance with the Former Studies

Due to low correlation between the SPAD value and reflectance in visible light bands, and high correlation at 760 nm, and 810 nm (Table 1), leaf cell structure was principal factor of effect on reflectance in above bands, which was in accordance with the former studies. Bauerle *et al.* [30] pointed out that although there was significant between the SPAD value measured and leaf transmission and absorption, there was no high correlation between the SPAD value and reflectance. Yang *et al.* [29] also indicated that the leaf thickness was important factor of effect on SPAD estimating model. Thus, we thought about not only visible light band, but also near infrared band in the reflectance-based SPAD estimating.

In addition, the nitrogen is important component of cell structure, thus changes in the disease leaf nitrogen content can affect its cell structure, and these changes can be reflected in spectrum reflectance, further in correlation with SPAD value.

5 Conclusions

This study screened out the reflectance-based bands with large difference between the disease and the normal samples, found the identifying bands with the same character through comparison of the reflectance for canopy in field and the reflectance for leaf in black background, the optimum growth period for reflectance-based rapeseed

leukoplakia identifying was form the 11th day after early anthesis to the 9th day after end anthesis, and 760–1080 nm was sensitive band in field identifying.

The correlation between the DI, the moisture content, the nitrogen content, and the SPAD value and reflectance in many character bands were analysed, and the multiple linear regression equations between the DI, the moisture content, the nitrogen content, and the SPAD value and reflectance were set up.

The same disease samples, rapeseed leukoplakia, and different disease samples, rapeseed virus, were used to validation of stepwise regression models, which made disease identifying precision raise.

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References

1. National Bureau of Statistics of China (2009). <http://www.stats.gov.cn/tjsj/qtsj/gjsj/>. Accessed 26 Sept 2013. (in Chinese with English Abstract)
2. Zhang, L.: The pollution of agricultural environment with chemical pesticide and its control. *J. Nanjing Agric. Technol. Coll.* **17**(4), 36–38, 43 (2001). (in Chinese with English Abstract)
3. State Environmental Protection Administration, China's Pesticide Pollution and Its Being Problems and Suggestions, *Environment Protection*, vol. 6, pp. 23–24 (2001). (in Chinese with English Abstract)
4. Zhu, M.Y., Zhang, J.X., Lu, Z.H., Wu, J.F.: Controlling the environmental pollution of chemical pesticide carrying out consecutions protection. *Jiangxi Plant Prot.* **26**(3), 29–31 (2003). (in Chinese with English Abstract)
5. Tu, Y.Q.: Thinking of problems of agriculture chemicals and circumstance. *Reclaiming Rice Cultiv.* **1**, 58–60 (2003). (in Chinese with English Abstract)
6. Wang, W.H.: Review for space optical remote sensing technology. *Inf. Syst. Eng.* (1), 159–141 (2012). (in Chinese with English Abstract)
7. Xue, L.H., Cao, W.X., Luo, W.H., Jiang, D., Meng, Y.L., Zhu, Y.: Diagnosis of nitrogen status in rice leaves with the canopy spectral reflectance. *Scientia Agricultura Sinica* **36**(7), 807–812 (2003). (in Chinese with English Abstract)
8. Xue, L.H., Cao, W.X., Luo, W.H., Zhang, X.: Correlation between leaf nitrogen status and canopy spectral characteristics in wheat. *Acta Phytoecologica Sinica* **28**(2), 172–177 (2004). (in Chinese with English Abstract)
9. Zhang, X.J., Li, M.Z., Zhang, Y.E., Zhao, P., Zhang, J.P.: Estimating nitrogen content of cucumber leaf based on solar irradiance spectral reflectance in greenhouse. *Trans. Chin. Soc. Agric. Eng.* **20**(6), 11–14 (2004). (in Chinese with English Abstract)
10. Li, J.M., Ye, X.J., Wang, Q.L., Zhang, C., He, Y.: Development of prediction models for determining N content in citrus leaves based on hyperspectral imaging technology. *Spectrosc. Spectr. Anal.* **34**(1), 212–216 (2014). (in Chinese with English Abstract)
11. Hou, X.J., Jiang, G.Y., Bai, L., Liu, S.J., Li, F.: Relationships between leaf nitrogen content and canopy reflected spectral in cotton. *Xinjiang Agric. Sci.* **45**(5), 776–781 (2008). (in Chinese with English Abstract)

12. Tian, Y.C., Cao, W.X., Jiang, D., Zhu, Y., Xue, L.H.: Relationship between canopy reflectance and plant water content in rice under different soil water and nitrogen conditions. *Acta Phytocologica Sinica* **29**(2), 318–323 (2005). (in Chinese with English Abstract)
13. Tian, Y.C., Zhu, Y., Cao, W.X., Dai, T.B.: Relationship between canopy reflectance and plant water status of wheat. *Chin. J. Appl. Ecol.* **15**(11), 2072–2076 (2004). (in Chinese with English Abstract)
14. Zhang, X.D., Mao, H.P., Zuo, Z.Y., Gao, H.Y., Zhou, Y.: Study on estimation model for rape moisture content under water stress based on hyperspectral remote sensing. *J. Anhui Agric. Sci.* **39**(30), 18451–18452+18487 (2011). (in Chinese with English Abstract)
15. Mao, H.P., Zhang, X.D., Li, X., Zhang, Y.: Model establishment for grape leaves dry-basis moisture content based on spectral signature. *J. Jiangsu Univ.: Nat. Sci. Ed.* **29**(5), 369–372 (2008). (in Chinese with English Abstract)
16. Fu, Y.B., Fan, Y.M., Sheng, J.D., Li, N., Wu, H.Q., Li, M.T., Li, L., Zhao, Y.: Study on relationship between alfalfa canopy spectral reflectance and leaf water content. *Spectrosc. Spectr. Anal.* **33**(3), 766–769 (2013). (in Chinese with English Abstract)
17. Xie, X.J., Shen, S.H., Li, Y.X., Li, B.B., Cheng, G.F., Yang, S.B.: Red edge characteristics and monitoring SPAD and LAI for rice with high temperature stress. *Trans. Chin. Soc. Agric. Eng.* **26**(3), 183–190 (2010). (in Chinese with English Abstract)
18. Zheng, F., Yue, J.Q., Shao, Y.H., Wang, Q.C., Zhang, G.T., Li, C.H.: Relationship between NDVI values and SPAD readings in different spring wheat varieties. *J. Triticeae Crops* **28**(2), 291–294 (2008). (in Chinese with English Abstract)
19. Chen, Z.Q., Wang, L., Bai, Y.L., Yang, L.P., Lu, Y.L., Wang, H., Wang, Z.Y.: Hyperspectral prediction model for maize leaf SPAD in the whole growth period. *Spectrosc. Spectr. Anal.* **33**(10), 2838–2842 (2013). (in Chinese with English Abstract)
20. Zou, H.Y., Ding, L.X.: Study on estimation model of tea leaf SPAD value based on reflective spectra data. *Remote Sens. Inf.* **5**, 71–75 (2011). (in Chinese with English Abstract)
21. Li, M.X., Zhang, L.S., Li, B.Z., Zhang, H.Y., Guo, W.: Relationship between spectral reflectance feature and their chlorophyll concentrations and SPAD value of apple leaves. *J. Northwest Forest. Univ.* **25**(2), 35–39 (2010). (in Chinese with English Abstract)
22. Cao, H.X., Zhang, C.L., Li, G.M., Zhang, B.J., Zhao, S.L., Wang, B.Q., Jin, Z.Q.: Researches of simulation models of rape (*Brassica napus* L.) growth and development. *Acta Agronomica Sinica* **32**(10), 1530–1536 (2006). (in Chinese with English Abstract)
23. Evers, J.B., Vos, J., Fournier, C., Andrieu, B., Chelle, M., Struik, P.C.: An architectural model of spring wheat: evaluation of the effects of population density and shading on model parameterization and performance. *Ecol. Model.* **200**, 308–320 (2007)
24. Zhang, R.M.: Changes in leaf dynamic after early anthesis for *Brassica Napus*. *Tillage Cultiv.* **5**, 59–60 (1988). (in Chinese with English Abstract)
25. Wang, J., Han, D.W., Ren, G., Guo, J.Q., Zhang, Y.S., Wei, C.Z., Song, Y.M.: A study on relation between SPAD value, chlorophyll nitrogen content cotton. *Xinjiang Agric. Sci.* **43**(3), 167–170 (2006). (in Chinese with English Abstract)
26. Yu, Y.L., Jia, W.K., Wang, C.H., Jiang, B.W.: Study on the relationship of SPAD readings to nitrogen content and to yield in spring maize. *J. Maize Sci.* **19**(4), 89–92+97 (2011). (in Chinese with English Abstract)
27. Hu, H., Bai, Y.L., Yang, L.P., Lu, Y.L., Wang, L., Wang, H., Wang, Z.Y.: Diagnosis of nitrogen nutrition in winter wheat (*Triticum aestivum*) via SPAD-502 and GreenSeeker. *Chin. J. Eco-Agric.* **18**(4), 748–752 (2010). (in Chinese with English Abstract)
28. Jiang, J.B., Chen, Y.H., Huang, W.J.: Study on hyperspectra estimation of pigment contents in canopy leaves of winter wheat under disease stress. *Spectrosc. Spectr. Anal.* **27**(7), 1363–1367 (2007)

29. Yang, H.Q., Yao, J.S., He, Y.: SPAD prediction of leave based on reflection spectroscopy. *Spectrosc. Spectr. Anal.* **29**(6), 1607–1610 (2009). (in Chinese with English Abstract)
30. Bauerle, W.L., Weston, D.J., Bowden, J.D.: Leaf absorptance of photosynthetically active radiation in relation to chlorophyll meter estimates among woody plant species. *Sci. Hortic.* **101**, 169–178 (2004). (in Chinese with English Abstract)