# 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 760-1080nm. 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 460nm, 550nm, 650 nm, 710 nm, 760nm, 1480 nm, and 1600 nm, between the leaf nitrogen content and the reflectance in 810nm, 870nm, 1080nm, 1280nm, 1320nm, 1540 nm, 1600 nm, 1650nm, and 1700nm, 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.

# 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 hydragric 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 breed 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 $P_2O_5$ ha$^{-1}$, and 90 kg $K_2O$ 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 1700nm) 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℃, then at 80℃ until reaching a stable weight, where dry weight were weighed using a 0.001g 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 cm$^2$ 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:

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

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

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

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

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

with $i$ = sample number, $n$ = total number of measurements, n-1= n when n≥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 810nm) 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 (760-1080nm) for healthy leaves was higher than that of the leukoplakia disease, while there was a reverse status in short-wave infrared band (1080-1700nm) except for around 1500nm 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 (460-760nm) for leukoplakia disease and healthy leaves (shown in Fig. 1 to Fig.4).
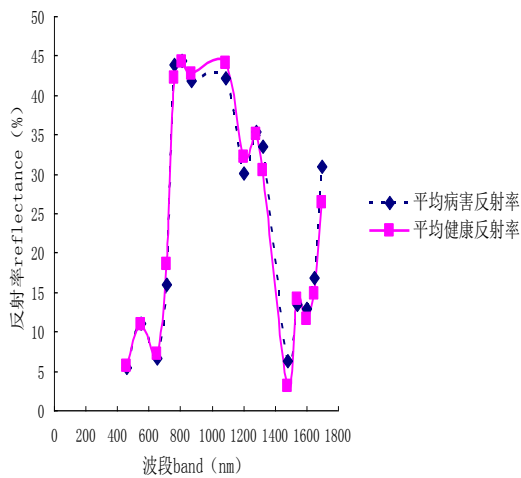
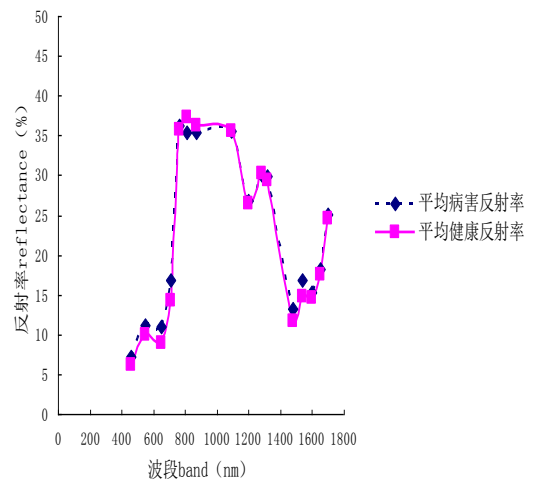**Fig.1.** Reflectance spectrum of *Brassica napus* leaves under the background of field groups anthesis (4.2)



**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)
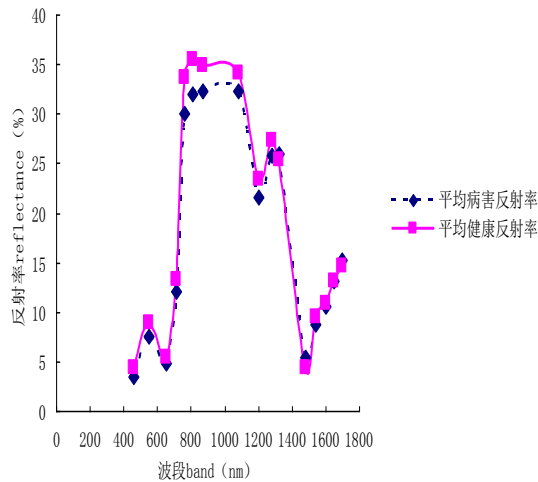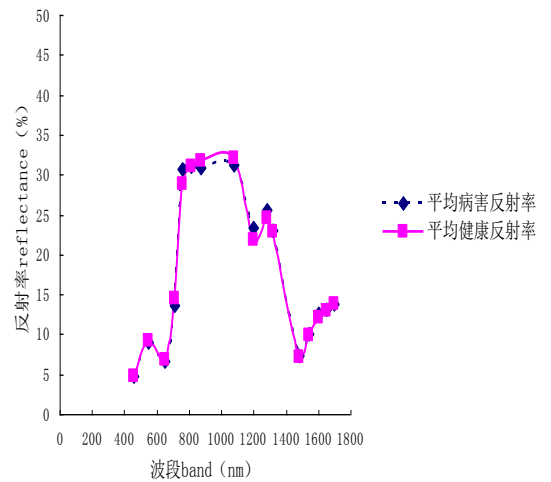


**Fig.4.** Reflectance spectrum of Brassica napus leaves under the background of field groups 17 days after finish flowering (5.3)

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 made under black background (shown in Fig. 5 to Fig.7), the results showed that the spectrum reflectance in visible light band (460-760nm) for healthy leaves less than that of the leukoplakia disease, and that of the reasons 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 (760-1080nm), 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 weaken 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.
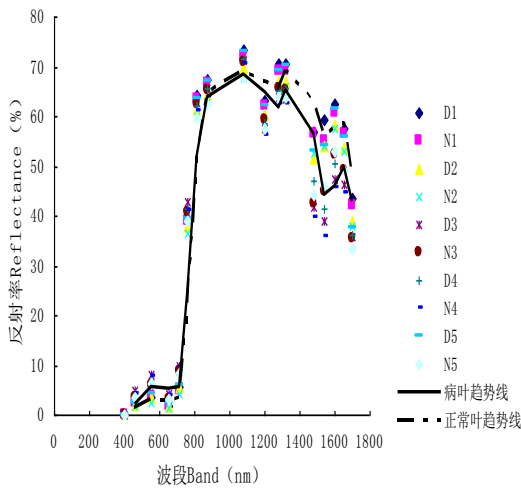


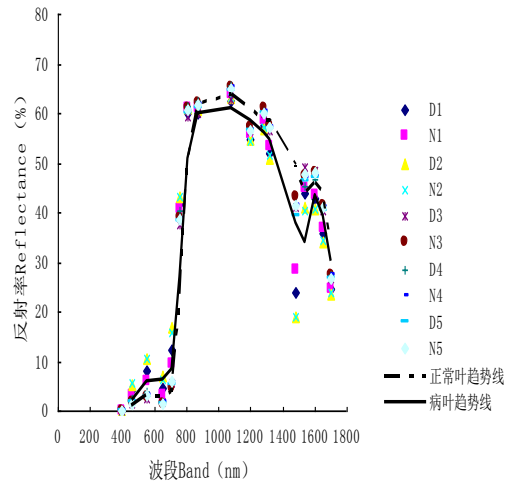**Fig.5.** Leaf reflectance under natural light in early anthesis (4.2)

**Fig.6.** Leaf reflectance under natural light 1 day after finish flowering (4.17)
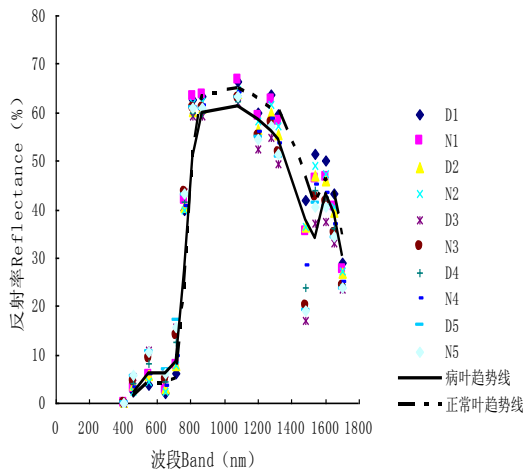
**Fig.7.** Leaf reflectance under natural light 9 days after

finish flowering (4.25)

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.

## 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 1200nm, 1480nm, and 1600nm was significant with $p<0.05$, that of between the reflectance and the disease leaf moisture content in short-wave infrared band 1320nm to1650 nm was significant with $p<0.01$, in which there was the highest negative correlation, -0.810, at 1540nm, that of between the reflectance and the disease leaf nitrogen content in band 810 nm to1320 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 $p<0.01$.

**Table1. 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** |
| 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 Fig. 8 to Fig. 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.
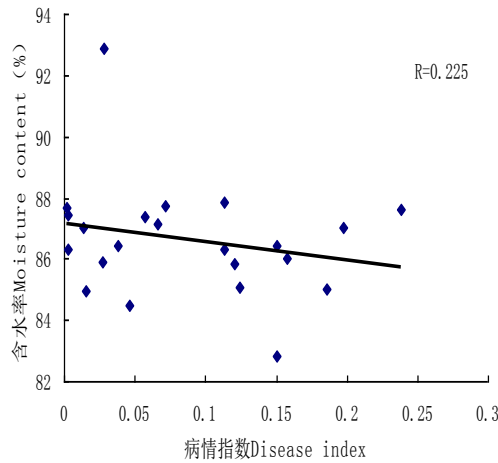


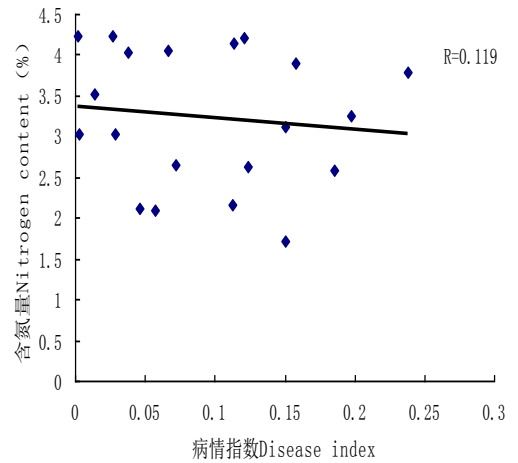**Fig.8.** Relationship between moisture content and DI



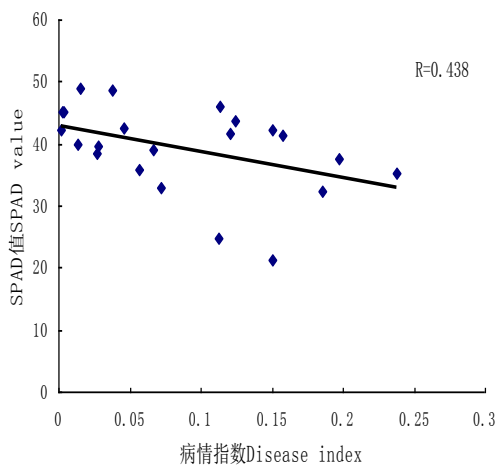**Fig.9.** Relationship between nitrogen content and DI



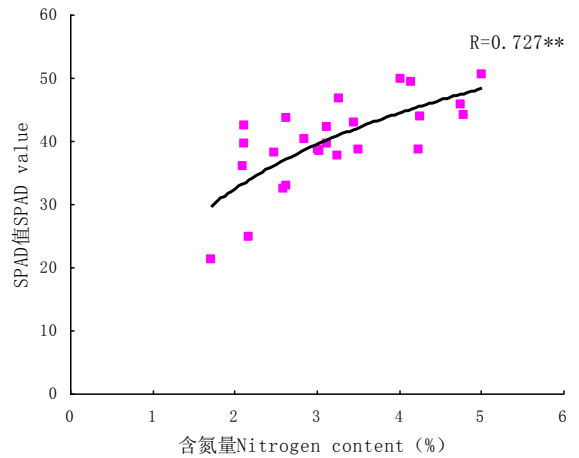**Fig.10**. Relationship between SPAD value and DI



**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

3.3.1.1 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 1540nm (Eq.1 in Table 3), for the disease leaf nitrogen content taking the reflectance at 810nm (Eq.2 in Table 3), and for the disease leaf nitrogen content taking the reflectance at 1280nm, 1080nm, and 650nm 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.

**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 650nm, 810nm, 1080nm, 1280nm, and 1540nm, 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 |

3.3.1.2 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 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 |

3.3.1.3 Principal components analysis. We selected the higher correlation bands with moisture content (460nm, 550nm, 650nm, 710nm, 760nm, 1480nm, and 1600nm), nitrogen content (810nm, 870nm, 1080nm, 1280nm, 1540nm, 1650nm, and 1700nm), and the SPAD value (1200nm, 1280nm, 1320nm, and 1540nm) 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 |
| | -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 (Table 8 and Table 9). The 1:1 figure on simulation and observation in the moisture content, the nitrogen content, and the SPAD value are represented in Fig. 12-16. 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**

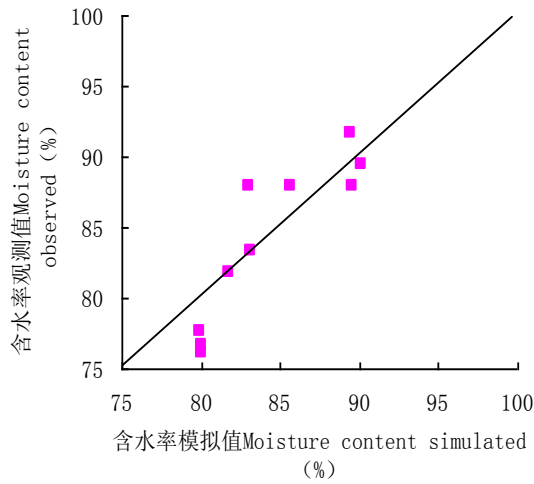| Parameters | N | $d_a$ | $d_{ap}$(%) | r | t | Sig. |
|---|---|---|---|---|---|---|
| SPAD | 5 | 22.684 | 52.901 | -0.512 | 7.247 | 0.002 |
| moisture content | 10 | 3.477% | 4.138 | 0.810 | -1.137 | 0.269 |
| nitrogen content | 10 | 3.424% | 85.465 | 0.748 | 0.520 | 0.616 |

**Fig.12.** Comparison of observation and simulation in moisture content of the same disease
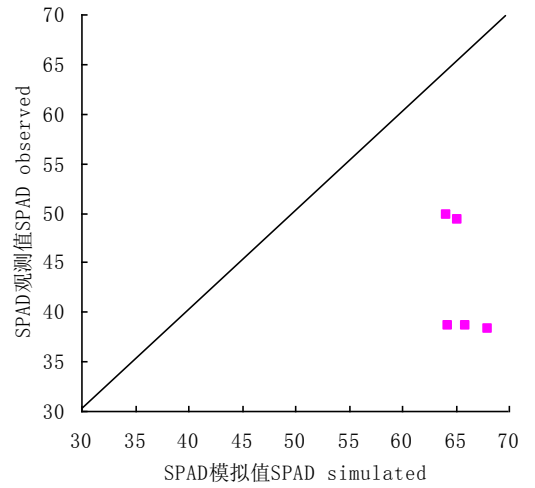
**Fig.13.** Comparison of observation and simulation in SPAD value of the same disease
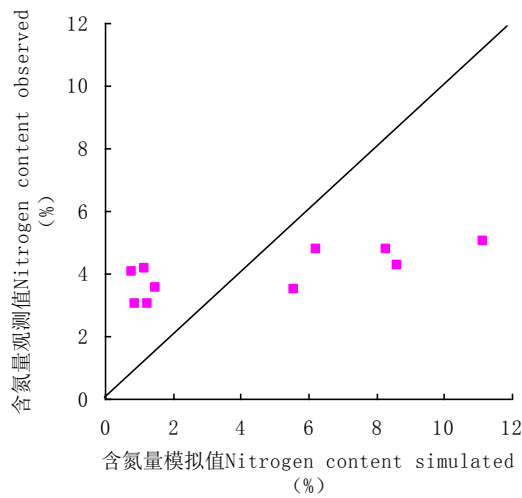
Fig.14  Comparison  of  observation  and  simulation  in
nitrogen content of the same disease

**Table 9. Stepwise regression model test of different diseases samples**

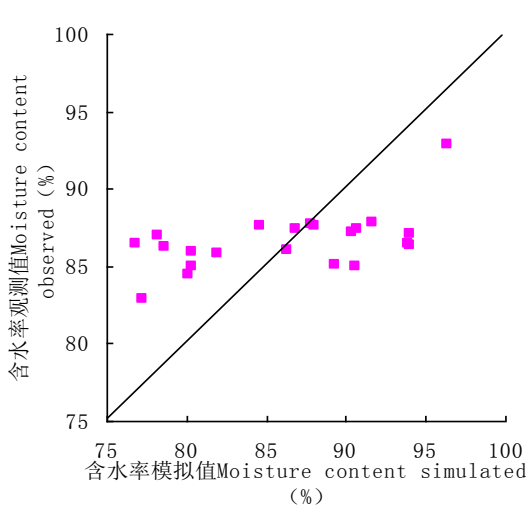| Parameters | N | $d_a$ | $d_{ap}(\%)$ | r | t | Sig. |
|---|---|---|---|---|---|---|
| SPAD | 21 | 20.260 | 76.849. | 0.775 | -12.934 | 0.000 |
| moisture content | 21 | 5.268% | 6.083 | 0.537 | 0.281 | 0.781 |



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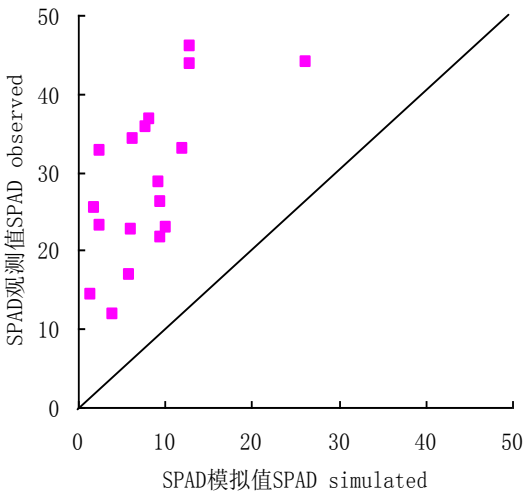content of different diseases



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 (1480nm), 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 exclude 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 760nm, and 810nm (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*.[29] 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*. [30] also indicated that the leaf thickness was important factor of effect on SPAD forecast model. Thus, we thought about not only visible light band, but also near infrared band in the reflectance-based SPAD forecast.

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 760-1080nm 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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