Determination of Corn Nutrient Status under N&K Stressed Condition Using Hyperspectral Analysis

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Abstract: Variable fertilization for crops, like corn, depends on monitoring nutrition condition. Thus, hyperspectral reflectance was used to predict chlorophyll and total nitrogen content under different N,K treatments during corn growth. The area of the experimental field was divided into 3 strips with different nitrogen treatment (N1:0 kg/ha–low, N2:314 kg/ha–normal, and N3:653 kg/ha–high). In each strip, there were 3 repetitions of 3 potassium treatments(K1:0 kg/ha–low, K2:214 kg/ha–normal, and K3:500 kg/ha–high). The results show that growth stages happened in advance under low nitrogen treatment and reflectance intensity, total N, and chlorophyll content are all largest under N2K1 in shooting stage. GNDVI(R2=0.88, RMSE=0.08) performs well for chlorophyll prediction under N3K2. In addition, MLR has the potential of determination of chlorophyll(R2=0.94, RMSE=0.02) and total N(R2=0.97, RMSE=0.09) in trumpet stage, as well as PLSR for chlorophyll(R2=0.99, SEC=0.01;SEP=0.09) and total N (R2=0.96, SEC=0.11;SEP=0.47) in trumpet stage.

Keywords: Hyperspectral Reflectance, Corn, Nutrient, MLR, PLS

1 Introduction

The corn (*Zea mays*) is cultivated large scale as significant food and economic plant. More inorganic fertilizers are undertaken to gain higher yield in conventional way, however it has been proved that excessive nitrogen(N) fertilizer will reduce the yield and harm environment. So, it is important to give growers a reasonable advice based on growth nutrition condition.

Since chlorophyll content is related to response to nutrition condition, the traditional fertilization is executed based on observing leaf color. But, in fact, this method is not reliable because growth is under complex conditions. Compared to chemical analysis, optical method is nondestructive and fast. Near infrared spectral technology has been used widely in past decades and proved the potential of prediction of growth condition[1-7]. Tang shows that the contents of chlorophyll and carotenoid are corresponded to spectra[8]. Cheng reports LAI and Chlorophyll can be predicted by the first derivative spectra of corn canopies[9].

Different treatment of nitrogen(N) and potassium(K) can induce growth nutrition change. This paper described the changes of total nitrogen and chlorophyll content,

tested by chemical method, in corn leaves under different match of N and K, then studied how to use hyperspectral analysis to predict total N and chlorophyll content. Wang reported that potassium(K) could affect the spectra of corn leaf[10]. Walburg considered that ratio of NIR to red was more advantage than red in N detection[3]. Cheng found some sensitive wavelength regions in different growth stages under different nutrient treatments (N,P, and K) [9] .

2 Materials and methods

2.1 Experiment design

Nongda 86 cultivar was selected as experimental corn and was sowed on April 25, 2009 at Shangzhuang Experimental Station of China Agricultural University, Beijing. Fertility of soil was detected before fertilization control. The area of the experimental field was about 12 ha and was divided into 3 strips with different nitrogen treatment (N1:0 kg/ha–low, N2:314 kg/ha–normal, and N3:653 kg/ha–high). In each strip, there were 3 repetitions of 3 potassium treatments(K1:0 kg/ha–low, K2:214 kg/ha–normal, and K3:500 kg/ha–high). So the total number of samples was 54. In addition, water and other fertilizers were treated under normal condition。

2.2 Spectra

Spectra was obtained under no wind condition between 10:00 and 13:00 and the dates were related to corn growth stages(Table 1). Trumpet and anthesis-silking stage were illustrated in Figure 1a and 1b respectively.

Table 1. Growth stages of corn (2009)

Stage No.	1	2	3	4	5	6	7
Name	Seedling		Shooting		Trumpet	Anthesis-silking	Filling
Date	5-27	6-02	6-11	6-23	6-30	7-07	7-15

(a)　　　　　　　　　　　　　　　　　(b)

Fig. 1. Images of (a) Trumpet stage and (b)Anthesis-silking stage

FieldSpec spectrometer (ASD Co., USA) was used to obtain reflectance spectra which covered from 325 to1075nm with 1nm resolution. Spectra were collected in the

middle part of the fourth(up to down) corn leaves. Three repetitions for each leaf were averaged for data processing. The standard white board was used as reference for each 3 leaves by equation (1) :

$$R = (R_S - Dark) / (R_W - Dark) , \qquad \textbf{(1)}$$

where R_S is reflectance of corn leaf; R_W is reflectance of the standard white board; and Dark means that no light enters the spectrometer. Considered that nitrogen may shift in filling stage, data was sampled from ear leaves.

2.3   Determination of chlorophyll and total nitrogen content

The same leaves were packaged with plastic bags for determination of chlorophyll and total nitrogen content after spectra collection. Chemical experiment and SP-2102 UV spectrophotometer (Cany Precision Instruments Co., Ltd, Shanghai China) were used together for chlorophyll content. After veins in leaf were removed, leaf was cut into small fragments. Then 0.4g fragments and 25ml solution with 1 for 95% ethanol and 2 for 99% acetone were mixed in a test tube. 24 hours later, the chlorophyll-containing solution was put into the spectrophotometer and A645, A652 and A663 readings were recorded for calculate the chlorophyll content by equation (2):

$$C_T = 20.29A645 + 8.05A663 \ or \ C_T = 20.29A652 , \qquad \textbf{(2)}$$

where C_T is a response value to total chlorophyll content（$mg \cdot L^{-1}$）。 The equation (3):

$$Chl = C_T \cdot V \times 10^{-3} / W , \qquad \textbf{(3)}$$

was used calculate chlorophyll content Chl($mg \cdot g^{-1}$), where V is volume of extract liquor，25ml；W is fresh weight of leaf，0.4g。

To total nitrogen detection, rest fragments were dried in oven and grinded into powder. 0.4g powder and 6.2g $CuSO_4 \cdot 5H_2O$ were mixed, then reacted with 60ml concentrated sulfuric acid in a tube. The tube was heat at 500℃ for 2 hours, then was cooled. Kjeldahl (FOSS NIRSystems, Inc., US) was used to determinate total nitrogen content($mg. g^{-1}$) of the leaf in solution.

2.4   PLSR analysis and multiple linear regression

Log (1/R) was often used as absorbance for spectral preprocessing. First and second derivative were also taken into eliminate the baseline drift error. Compared the derivative spectra of high and low total N content, we selected a set of G, R, NIR wavelengths. Then single-band spectra and some VIs(Vegetable Indexes), like red edge slope, NDVI, GNDIV, DVI, RVI and SVI, were used to predict chlorophyll and total nitrogen content.

MLR(Multi Linear Regression) and PLSR (PLS Regression) were also introduced

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to determinate chlorophyll and total nitrogen content in this paper, which were all multivariate data processing methods and fit to hyper spectra. PLS (Partial Least Squares) was based on MLR and PCA (Principle Component Analysis), and PCA had the potential for dimension reduction under considerable variables. So, PLSR was more powerful to calibrate spectrum data than MLR. However, Over-fitting can happen to validation sometimes. In this paper, MATLAB (Math Works Inc., Natick, MA, USA) was used for data processing tool。

3 Result and Discussion

3.1 Chlorophyll and total nitrogen content

Figure 2 and 3 show chlorophyll and total N content under different N treatments in different growth stages respectively. Chlorophyll content increased early, then decreased. Total N content had the same change as Chlorophyll, but increased again in filling stage(July 15) for the reason that corn steps into reproductive growth.

To low nitrogen treatment, the chlorophyll content reach highest in early shooting stage(June, 11) , and the total content decreased at shooting stage(June 23). The same growth stages happen in advance for low nitrogen treatment. Under low nitrogen treatment, the high N content in filling stage also proved that grain is difficult to get more nitrogen nutrient. In addition, Figure 2 and 3 indicated that the total N content under high N treatment deceased later than chlorophyll content, and later than normal N treatment.
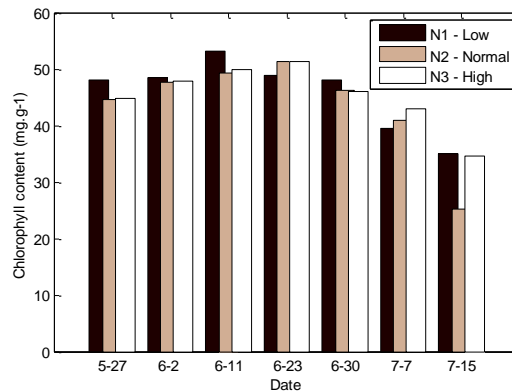

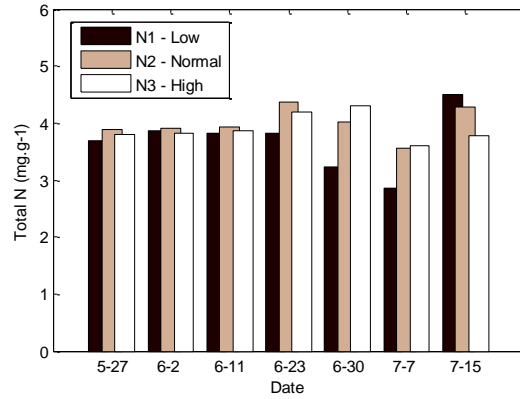**Fig. 2.** Chlorophyll content in each stage under different nitrogen treatments

Fig. 3. Total N content in each stage under different nitrogen treatments

Different NK treatments were studied in-depth except low nitrogen treatment, which showed growth ahead and poor performance of nitrogen absorption in filling stage based on the above analysis. Figure 4 and 5 show that total N and chlorophyll content of leaves under N2K1 in the shooting stage are highest. Followed by N3K3 and other normal N, the lowest under N3K2 and N3K1. It can be concluded that normal nitrogen treatment is fit to low potassium and high nitrogen needs high potassium.
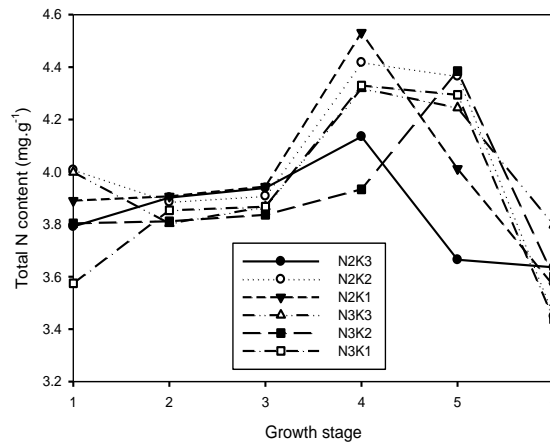

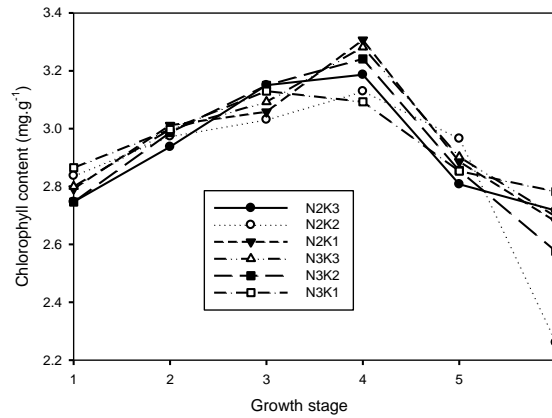**Fig. 4.** Total N content in each stage under different N,K treatments

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Fig. 5. Chlorophyll content in each stage under different N,K treatments

3.2 Correlation between total N content and chlorophyll content

Total nitrogen content and chlorophyll content of leaf showed low correlation at different growth stages. In Figure. 6 and 7 , the top two performances happened in shooting stage (R^2 = 0.83; RMSE = 0.10) with positive correlation and trumpet stage (R^2 = 0.69 ; RMSE = 0.27) with negative correlation.
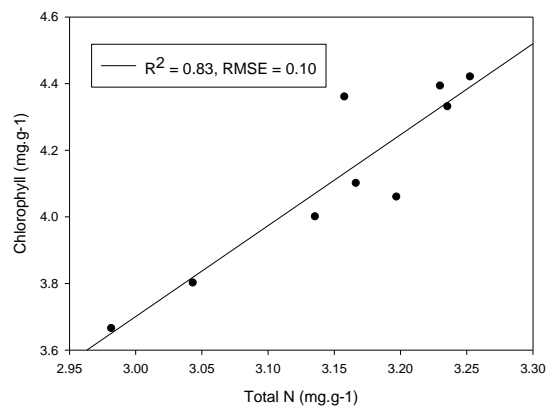

Fig. 6. Correlation between total N and chlorophyll at Shooting stage (June 23)
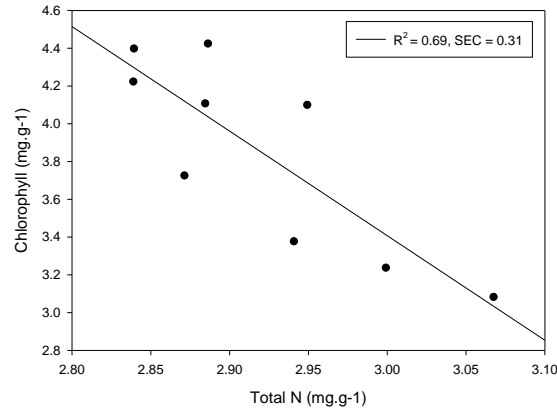
Fig. 7. Correlation between total N and chlorophyll at Trumpet stage (June 30)

The correlation coefficient between total N content and chlorophyll content was given in Table 2, excluding filling stage where they are significant different. Three treatments, where the correlation was higher than 0.8, are N1K3(R2 = 0.83; RMSE = 0.15), N2K1(R2 = 0.81; RMSE = 0.12), and N2K3(R2 = 0.81; RMSE = 0.07). Under low N,K treatment, although growth stage was earlier, the correlation of chlorophyll and total N was higher. In contrast, high nitrogen treatments had low correlation, which indicated that excess nitrogen would affect transform nitrogen to chlorophyll.

Table 2. Correlation between total N and Chlorophyll under different treatments

N,K treatment	R2	RMSE
N1K3	0.83	0.15
N1K2	0.78	0.19
N1K1	0.31	0.32
N2K3	0.81	0.07
N2K2	0.67	0.19
N2K1	0.81	0.12
N3K3	0.24	0.18
N3K2	0.06	0.23
N3K1	0.22	0.29

3.3   Leaf spectrum

Typical spectra of corn leaves (Anthesis-silking stage; The sample S5, total N = 4.1234%; S26, total N = 2.0707%) are illustrated in Figure. 8. Wavelength ranges below 405 and above 978 were cut with high noise. In the visible region, most of the light was absorbed and there was a small peak in 553nm related to green band. The spectra curve jump to strong reflectance in near-infrared region through red edge (685~750nm), which can be related to the internal organizational structure of corn leaf.
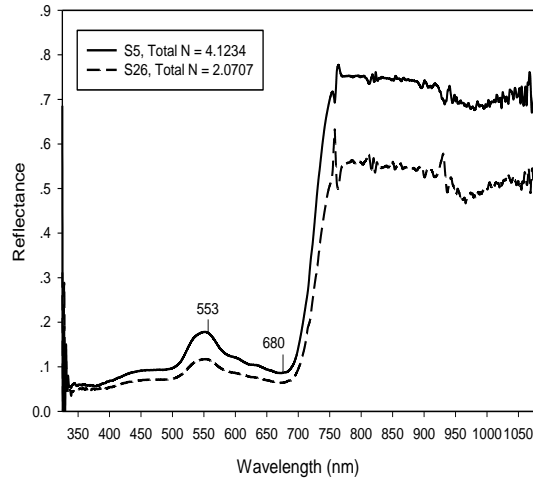
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Fig. 8. Reflectance spectra of two corn leaves with high and low total N content

Figure 9 and 10 show spectral reflectance at 553 and 818nm under different N, K treatments in different stages respectively. Overall, changes of reflectance intensity are like wave, and there are two peaks in the shooting stage(Stage 4) and anthesis-silking(Stage 6) stage. The reflectance intensity at 553nm is lowest in trumpet stage(Stage 5).

N2K1, N3K3 were more strong and N3K2, N2K3,N1K2 were lower in shooting stage at both wavelength. It was similar to the result of leaf total N content and chlorophyll content. Compared to other N,K treatments, the intensity of reflectance under N2K1, N3K2 change obviously in trumpet and anthesis-silking stage(Stage 6). High nitrogen treatments increased gradually, and low nitrogen treatment declined in general.
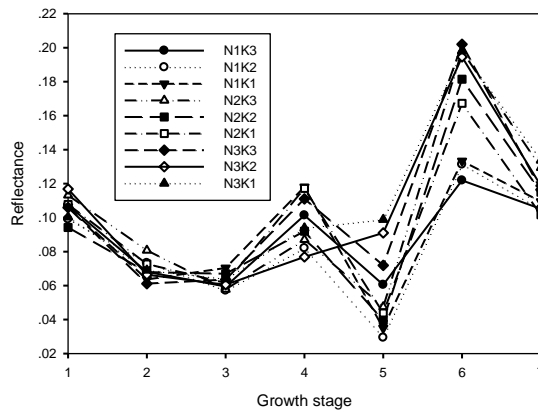

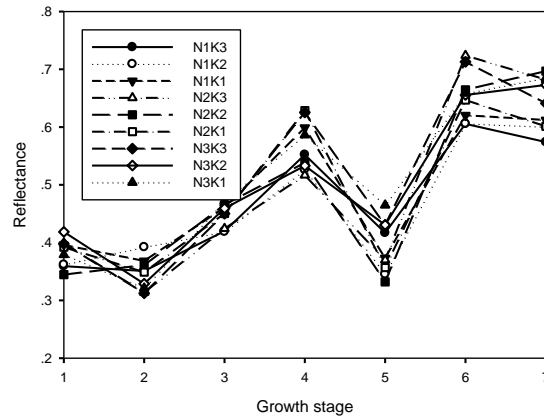**Fig. 9.** Reflectance in each stage at 553nm

Fig. 10. Reflectance in each stage at 818nm

3.4   Wavelength selection and VIs regression

Figure 11 shows Second derivative spectra of log(1/R) of the same corn leaves shown in Figure 8. 553,680,760 and 818nm were selected as green, red, red edge, and near-infrared wavelengths.

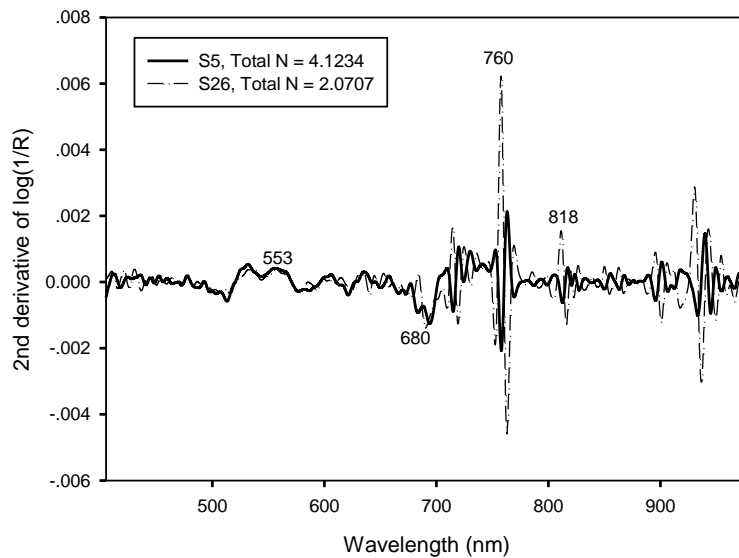**Fig. 11.** Second derivative spectra of log(1/R) of the same corn leaves shown in Figure 8
Single-band spectra and some VIs, like red edge slope, NDVI, GNDIV, DVI, RVI and SVI, were used to predict chlorophyll and total nitrogen content. The best performance was the correlation between GNDVI(R^2 = 0.88, RMSE = 0.08) and

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chlorophyll content under N3K2, followed by G (R^2 = 0.79, RMSE = 0.13) under N2K2 and NDVI (R^2 = 0.78, RMSE = 0.11) under N3K2.

To total N, all top 3 performances happened in anthesis-silking stage(Stage 6), including G (R^2 = 0.88, RMSE = 0.12), R (R^2 = 0.83, RMSE = 0.14) and GDNVI (R^2 = 0.81, RMSE = 0.15) respectively.

3.5 PLSR for total N and chlorophyll content

Some efficient results is shown in Table 3 by PLSR for total N and chlorophyll content in different growth stage. Better performances for Chlorophyll content were shooting stage (R^2 = 0.95, SEC = 0.03; SEP = 0.12) and trumpet stage (R^2 = 0.99, SEC = 0.01; SEP = 0.09) respectively. Better results for total N content were filling stage (R^2 = 1.00, SEC = 0.02; SEP = 0.58) and trumpet stage (R^2 = 0.96, SEC = 0.11; SEP = 0.47) respectively. Compare the four results, prediction for total N content is not perfect with high SEP in validation. In addition, The PLSR results for total N and chlorophyll content under different N,K treatment are good for calibration, but over-fitting for validation.

In general, total N and chlorophyll content can be predicted in trumpet and shooting stage using reflectance spectra for assessment of corn nutrition condition.

Table 3. PLSR for total N and chlorophyll content in different growth stage (5 factors)

| Stage | Chlorophyll content | | | | Stage | Total N content | | | |
| | Calibration | | Validation | | | Calibration | | Validation | |
	R2	SEC	R2	SEP		R2	SEC	R2	SEP
Shooting	0.95	0.03	0.71	0.12	Trumpet	0.96	0.11	0.72	0.47
Trumpet	0.99	0.01	0.70	0.09	Filling	1.00	0.02	0.53	0.58

3.6 MLR analysis for total N and chlorophyll content

The intensity of log(1/R) at R, G, NIR, and the slope of red edge were selected to implement MLR to predict total N and chlorophyll content. The result is described in Table 4. The best correlation ($R^{2)}$) for chlorophyll content prediction was 0.94 (RMSE = 0.02), and for total N content was 0.97 (RMSE = 0.09) in trumpet stage(June 30).

Table 4. MLR for N and chlorophyll content in different growth stage

Date	R^2 for Chlorophyll	SEC	R^2 for total N	SEC
5-27	0.72	0.07	**0.26**	0.13
6-02	0.54	0.02	0.66	0.04
6-11	0.79	0.05	0.89	0.02
6-23	0.75	0.05	0.78	0.13
6-30	**0.94**	0.02	**0.97**	0.09
7-07	0.51	0.14	0.92	0.10
7-15	**0.48**	0.23	0.60	0.21

Table 5 shows that the best correlation ($R^{2)}$) for chlorophyll content prediction is 0.96

(RMSE = 0.04) under N3K3 treatment, and for total N content is 0.99 (RMSE = 0.04)
under N3K1 treatment.

Table 5. MLR for N and chlorophyll content under different N,K treatment

N/K treatment	R^2 for Chlorophyll	SEC	R^2 for total N	SEC
N1K3	0.85	0.09	0.91	0.11
N1K2	0.78	0.17	0.81	0.17
N1K1	<u>**0.59**</u>	0.12	0.68	0.22
N2K3	0.61	0.12	<u>**0.50**</u>	0.12
N2K2	0.88	0.10	0.92	0.09
N2K1	0.80	0.09	0.76	0.14
N3K3	**0.96**	0.04	0.93	0.05
N3K2	0.94	0.06	0.85	0.09
N3K1	0.82	0.05	**0.99**	0.04

4 Conclusion

Chlorophyll and total N content increased early, then decreased until filling stage
when reproductive growth started. The correlation between Chlorophyll and total N
content was analyzed because growth stages happened in advance under low nitrogen
treatment. Chlorophyll and total N content under the treatments of normal nitrogen
and low potassium performed best in shooting stage, which means high nitrogen is
not necessary.

Reflectance under N2K1 and N3K3, similar to total N content and chlorophyll content,
were more strong than N3K2, N2K3,N1K2 in shooting stage at both 553 and 818nm
wavelength. GNDVI(R^2 = 0.88, RMSE = 0.08) was better than other VIs for
prediction of chlorophyll content under N3K2, as well as G (R^2 = 0.88, RMSE = 0.12)
was better for total N content prediction in anthesis-silking stage. In trumpet stage, the
correlation of Multiple Linear Regression(553, 680, 760 and 818) for prediction of
Chlorophyll and total N content are 0.94(RMSE = 0.02) and 0.97 (RMSE = 0.09)
respectively, and PLSR also had good performance for total N content (R^2 = 0.96,
SEC = 0.11; SEP = 0.47) and chlorophyll content (R^2 = 0.95, SEC = 0.03; SEP =
0.12). In a word, total N and chlorophyll content can be predicted in trumpet and
shooting stage or some N,K treatments using reflectance spectra for assessment of
corn nutrition condition.

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