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Nutrients Change and Spectral Response of Spring Corn Leaf for Varying Amounts of Potassium Fertilization

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Abstract: The application of spectral properties as a diagnostic tool in nutrient deficiency diagnosis requires further understanding of the relationship between spectral properties and nutrient concentration in plant tissue. Therefore, to examine the effect of variable potassium fertilization on corn (Zea mays L.) leaves and the response on leaf spectral reflectance. We designed an experiment to examine the effect variable amounts of potassium fertilization during four stages of corn growth. Our results showed that the difference in potassium leaf content is significant at the 0.05 probability level with increasing potassium fertilization. We then examined the correlation between corn leaves spectra reflectance and nutrient content as a function of variable levels of potassium fertilization over the entire corn growing season by potted plant experiment. Digital models were constructed to assess potassium content in leaves by utilizing leaves spectral reflectance in the booting stage. Our results showed that there was a correlation between the spectral reflectance of corn leaves and corresponding leaf inner moisture, chlorophyll and other nutrient content including nitrogen, phosphorous, potassium, calcium, magnesium, copper, iron, manganese, and zinc. These differences in the spectral reflectance held for the entire growing season. 730—930 nm and 960—1100 nm were defined as sensitive wave bands to assess potassium nutrition condition in booting stage, and spectral variables including $R_{767}+R_{1057}$, $(R_{767}+R_{1057})/(\log R_{767}+\log R_{1057})$ and $(R_{767}-R_{1057})/(\log R_{767}-\log R_{1057})$ could predict accurately corn leaf potassium-content in the booting stage. Our analysis indicated a correlation between various components in the inner leaves and spectral reflectance at this stage. Inflection points occurred at 410nm, 550nm, 710nm, 950nm in every curve. When correlations between the various components were high, their spectra correlation curves were coincident or symmetrical.

Key words: Spring corn; potassium fertilization; nutrients; spectral reflectance; correlation
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1 INTRODUCTION

Applications for spectral technology have become increasingly important in agriculture. Hyperspectral technology has the advantages of more numerous wave bands and higher spectral resolution and it therefore is important in detecting plant canopy and the physical structure and chemical component of leaves. Several authors have found empirical relationships between leaf spectral properties and leaf morphological and physiological conditions, including leaf thickness[1], presence of pubescence[2], nitrogen (N) content[3-7], phosphorous (P) content[6,8], water content[9,10], and chlorophyll concentration[11,12].

However, use of spectral properties as a diagnostic tool in nutrient deficiency diagnosis requires further understanding of the relationship between spectral properties and nutrient concentration in plant tissue. Aspects of this relationship have been described for corn for varying N fertilizer levels[12,13].

Our objective was to examine the correlation between K content and spectral reflectance of corn leaves for varying K fertilizer concentrations over the entire

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growing season, in order to construct exact models for estimating K nutrition conditions. In addition, the correlation between moisture, chlorophyll, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) content and spectral reflectance of corn leaves were analyzed with varying K fertilizer concentration in the booting stage, in order to determine points of sensitivity for nutrients in the spectral signatures. The aim of the study was to establish a theoretical basis for the detection of nutrient deficiencies with spectral technique.

2 MATERIALS AND METHODS

Research was carried out from April to September in 2004 at the Chinese Academy of Agricultural Science. Adopting standard potted plant experiment design, we used pots (40cm deep and 30 cm in diameter) contained 12kg of natural drying soil (e.g. damp soil with 1.2% organic matter, 24.8mg/L available NH_4-N, 18.3mg/L available phosphate, and 43.5mg/L available K). We used corn (Zea mays L.) variety Nongda 108 in this experiment. Before the jointing stage, there were two plants in each pot. After the jointing stage, each pot contained only one corn plant. K as KCl was applied in five concentrations of 0kg/hm^2 K (K1), 150kg/hm^2 (K2), 300kg/hm^2 (K3), 450kg/hm^2 (K4), 600kg/hm^2 (K5). N as urea was applied in concentrations of 300 kg/hm^2 as 60% for preplanting basal, 15% for jointing dressing, and 25% for booting dressing, respectively. P as monocalcium phosphate [$Ca(H_2PO_4)_2$] was applied at a concentration of 150kg/hm^2. Other micronutrients were adjusted as needed. P, K, and micronutrients were applied and incorporated into the soil before sowing. The experiment was a randomized complete design with three replications for each K treatment. Hyperspectral reflectance measurements and sampling analysis were performed at jointing, booting, anthesis-silking and filling stage respectively.

Hyperspectral reflectance measurements were collected from 350 to 1100nm in 1.4nm intervals with the FieldSpec Pro (350—2500nm) spectoradiometer manufactured by Analytical Spectral Devices. The spectral resolution is 3nm at 700nm. All spectral measurements were made on cloudless or near cloudless days at 10:30 to 14:00 local time. Spectral data were acquired as digital numbers with a 25° field of view. We chose the sixth fully-expanded leaf to get spectral data for the jointing and booting stages, and the twelfth fully-expanded leaf (fruit leaf) for the anthesis-silking and filling stages. We analyzed each leaf twenty times to collect the spectral data. Before and after the same leaf spectral data acquired, the radiometer was calibrated before and after each analysis with a white reference panel. We aggregated the raw spectral data by taking the average of every five consecutive bands to reduce the volume of data. This reduction in data produced 215 spectral bands with a bandwidth of approximately 10nm.

Immediately following spectral sampling, we determined chlorophyll-(a+b), moisture, N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn content. Leaves were stored with dry ice for chl testing. Dirt was removed from the surfaces of the leaves with pledget and the main artery was eliminated. We then broke into pieces. Approximately 0.2g leaf samples were dipped in a 50ml ethanol (95%)-acetone (99%) (1:1v/v) mixture and stored in the dark for several days until the leaves became white. The absorptance (OD) of extracted liquid was measured at 663nm and 645nm on a 722 spectrophotometer. The results were calculated as follows:

$$C_A = 12.7OD_{663} - 2.59OD_{645} \quad (1)$$

$$C_B = 22.9OD_{645} - 4.67OD_{663} \quad (2)$$

$$C_{A+B} = 20.3OD_{645} + 8.04OD_{663} \quad (3)$$

where C_A, C_B, C_{A+B} represented for chl-a, chl-b, chl-(a+b) concentration respectively. The units are in mg/L.

$$chl(mg \cdot g^{-1}) = C * V/(W * 1000) \quad (4)$$

where C (mg/L) is chl concentration, V (ml) is the total volume of extracted liquid, and W (g) is the fresh weight of the leaf.

The fresh moisture content (FMC) was testing by using electrical drying method in oven. FMC was defined as the ratio between the quantity of water (fresh weight-dry weight) and the fresh weight:

$$FWC(\%) = (FW - DW)/FW \times 100 \quad (5)$$

where FW is the fresh weight measured before drying and DW the oven-dried weight of the same sample. For nutrient analysis, 0.5g samples were wet-ashed by the H_2SO_4-H_2O_2 digestion at 300℃. Ashed extracts were brought to 100ml volume. N was determined by distillation and titration. P was determined by vanadium-molybdenum yellow colorimetry. K, Ca, Mg, Cu, Fe, Mn, and Zn were determined by atomic absorption spectrometry.

3 RESULTS AND DISCUSSION

3.1 Analysis of spring corn leaf moisture, chl-(a+b), and nutrient content for the five K-fertilization concentrations and four corn development stages

Table 1 illustrates that spring corn leaf moisture, chl-(a+b), and other nutrients varied as a function of variable K-fertilization during the four stages of corn development. K content in the six fully-expanded leaf at the jointing and booting stages and the twelfth fully-expanded leaf at the anthesis-silking and filling stages gradually increased with increasing amounts of K fertilization. The difference between treatments was significant at the 0.05 probability level. The compositional changes in other elements were not as well ordered. At jointing stage, the concentrations of five elements including chl-(a+b), N, Ca, Mg and Mn decreased with increasing K fertilization; but moisture content increased. The relationship between other nutrients and increasing amounts of K fertilization are not clear. Ca, Mg, Mn, and moisture contents appear to behave the same in the booting as the jointing stage but Zn and Cu content decreased with increasing K fertilization. At the anthesis-silking stage, the

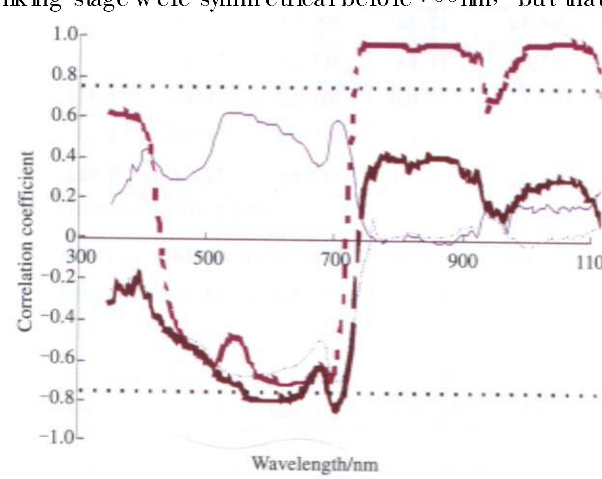
Table 1 Nutrients content change of corn leaves for the four development stages and the four K fertilizer treatments

	K/%	N/%	P/%	Ca/%	Mg/%	Zn/(mg/kg)	Mn/(mg/kg)	Cu/(mg/kg)	Fe/(mg/kg)	W/%	chl/(mg/g FW)
Jointing Stage (9 June)											
K1	0.73e	1.54a	0.23a	0.48a	0.69a	22.8a	52.6a	7.4bc	162.6a	69.4c	2.52a
K2	1.08d	1.57a	0.23a	0.43b	0.57b	19.0ab	46.1b	9.8a	177.1a	69.9bc	2.53a
K3	1.46c	1.36b	0.22ab	0.42bc	0.49c	18.5b	40.6c	8.8ab	156.2a	70.4c	2.11b
K4	2.21b	1.33b	0.22b	0.38d	0.37d	16.9b	36.7d	6.3cd	151.1a	72.4ab	2.14b
K5	2.60a	1.27b	0.23a	0.34cd	0.33e	17.5b	38.1d	5.3d	172.2a	73.1a	2.06b
Booting Stage (28 June)											
K1	0.17c	2.00a	0.12a	0.69a	0.76a	26.0a	64.1a	12.3a	309.2a	71.2b	3.02cd
K2	0.23b	1.82b	0.12a	0.69ab	0.73b	25.7a	57.9b	11.8a	317.2a	71.4b	2.97d
K3	0.27b	1.84b	0.12a	0.66a	0.65c	20.8b	51.6c	10.0ab	301.2a	71.8b	3.46b
K4	0.48a	1.74b	0.11b	0.65ab	0.53d	13.7c	52.4c	8.6b	310.9a	73.2ab	3.19c
K5	0.50a	1.87ab	0.11ab	0.63a	0.47e	15.9c	50.0c	9.7b	311.6a	74.6a	3.92a
Anthesis-silking Stage (19 July)											
K1	0.14e	1.63b	0.09d	0.79a	0.90a	19.3abc	73.0a	9.5bc	224.3a	67.7d	1.81b
K2	0.21d	1.56b	0.11c	0.83a	0.84b	22.0a	58.4b	8.0c	218.1ab	68.7bc	1.94b
K3	0.25c	1.82a	0.13b	0.69b	0.72c	18.9bc	57.5b	10.7b	207.3b	70.8cd	2.92a
K4	0.56b	1.86a	0.14ab	0.68c	0.60d	16.9c	54.1c	10.6b	162.8c	72.7ab	2.79a
K5	0.72a	1.85a	0.14a	0.62bc	0.56e	19.8ab	54.0c	13.3a	216.4ab	73.9a	1.89b
Filling Stage (15 August)											
K1	0.15e	1.58b	0.10c	1.07a	0.97a	42.3a	79.2b	15.5a	484.7a	69.0a	2.18c
K2	0.18d	1.61b	0.11bc	1.02b	0.93a	41.8a	88.9a	10.8b	311.8c	69.1a	2.63b
K3	0.29c	1.67b	0.13a	0.98b	0.88b	39.0ab	77.5c	11.7b	298.9c	69.3a	2.61b
K4	0.32b	1.87a	0.12a	0.93c	0.76c	38.0b	74.2d	12.5b	250.0d	70.8a	3.16a
K5	0.37a	1.76ab	0.12ab	0.87d	0.66d	37.4b	75.4d	11.8b	336.0b	71.3a	2.77b

change in nutrient contents for the fruit leaf and the sixth fully-expanded leaf was similar. P, Cu, and moisture content increased and Ca, Mg, Mn content decreased with increasing K fertilization. At the filling stage, moisture content decreased but Ca, Mg, Zn, Mn increased with increasing K fertilization. In summary, different amounts of K fertilization had a variable effect on corn root absorption and transport of other nutrients resulting in differences in overground leaf cell structure and elemental concentration. Therefore, we expect that these corn leaves will have a variable spectral response when irradiated by visible and NIR light.

3.2 Correlation between corn leaf spectral reflectance and leaf K content for the four development stages

Fig. 1 shows that through the four stages of corn development, the correlation between corn leaf spectral reflectance and K content varies across the entire spectrum from 300 to 1100nm. This correlation has a significantly positive relationship at 730—930nm and 960—1100nm in the booting stage and a significantly negative relationship at 570—650nm and 690—715nm in the filling stage. Note that the qualitative and quantitative correlation in the jointing stage and anthesis-silking stage were symmetrical before 760nm, but that



Notes: 1. —— ···· ---- ▬ ▬ represent the jointing stage, booting stage, anthesis-silking stage and filling stage, respectively.
2. The two dashed lines parallel to the x-coordinate axis denote significance at the 0.05 probabiligy level.

Fig. 1 Correlation between leaves spectral reflectance and K content in four stages

there is essentially no correlation at wavelengths greater than 760nm. All growing stages have a positive relationship from 760 to 1100nm, but the extent to which each stage is positive varies at wavelengths greater than 760nm. The best correlation at high wavelengths is between the booting and filling stages. We conclude that it is possible to assess K nutrition in corn leaves by analyzing spectral reflectance in the visible spectral region for the jointing, anthesis-silking, filling stages and the NIR (near-infrared) spectral region for the booting stage. Therefore, the appropriate spectral wave band should be chosen to asses corn leaf nutrition according to corn growing stage.

3.3 Construction of regression model between leaf spectral reflectance and leaf K content in the booting stage

According to the previous analysis, 730—930nm and 960—1100nm wavelengths are the best at assessing potassium nutrition condition in the booting stage. So, we selected 767nm and 1057nm from the two sensitive wave bands to perform a regression analysis in order to construct a single and dual wave band component spectral index. Our calculations showed that although the correlation between spectral properties at 767 and 1057nm and leaf K content were always significant at the 0.01 probability level (Table 2), the coefficient of variance (C.V = 0.38%) of the regression equation constructed by the single wave band spectral reflectance and leaf K content exceeded the coefficient of variance (C.V = 0.01%) of the dual wave bands spectral reflectance and leaf K content. In other words, the coefficient of variance of the regression equation constructed for the dual wave band component spectral index are relatively stable. We therefore conclude that it is possible to assess corn leaf K nutrition condition using dual wave band component spectral index. Perhaps the spectral indices from single wave bands were more easily affected by the angle of leaves or background reflectance. Daughtry et al. also found that attempts to assess plant N status based on canopy reflectance in a single band often were confounded by the variability in background reflectance and/or LAI[14]. So we conclude that a

Table 2 Comparison of single and dual wave band component spectral indices used to assess leaves K content in the booting stage

Spectral variables	Equation	Determine coefficient
R_{767}	y=0.0559x−2.311	R^2=0.9482
R_{1057}	y=0.058x−2.4221	R^2=0.9533
$R_{767}+R_{1057}$	y=0.0285x−2.3669	R^2=0.9511
$(R_{767}+R_{1057})/(\log R_{767}+\log R_{1057})$	y=0.1288x−3.3104	R^2=0.9509
$(R_{767}-R_{1057})/(\log R_{767}-\log R_{1057})$	y=0.0247x−2.3668	R^2=0.9511

spectral index based on multiple wave band component can estimate more accurately plant nutrition condition.

3.4 Correlation between leaf spectral reflectance and leaf chl-(a+b), moisture and nutrient content in the booting stage

Nutrient uptake is greatest during the booting stage. Corn growth is hypersensitive to many nutrients. The variation in K levels caused the differential absorption of nutrients by the corn root. This caused changes in the metabolism of the plant including alteration of the anabolic organic compound inner leaf. The change in the compound inner leaf caused the variation in the leaf spectral reflectance in the different bands. Accordingly, the correlation between leaf spectral reflectance and leaf chl-(a+b), moisture and several nutrients changed with this change. Fig. 2 shows that correlations between the sixth leaf spectral reflectance and its chl-(a+b), moisture and nutrient contents occur either in the visible or NIR spectral region in the booting stage. We hypothesize that the reason that corn metabolism increased was due to the increased assimilation of nutrients just as the inner leaf changed significantly at this stage. The correlation between the leaf reflectance and leaf chl-(a+b), moisture, nutrients contents have to be significantly improved beyond what is shown in Fig. 2 in order to pinpoint important changes in corn development.

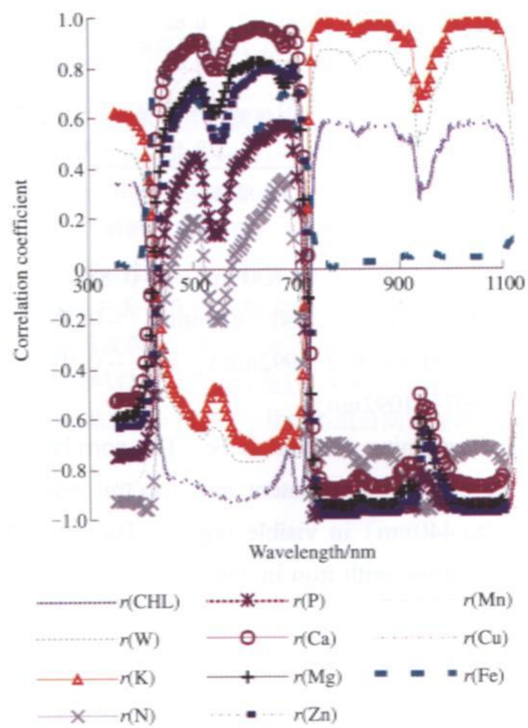


Fig. 2 Correlation curves for leaf spectral reflectance and leaf chl-(a+b), moisture and nutrient content in the booting stage

Nutrients were classified into three groups according to the correlation between spectral reflectance and corn development stage. The first group consisted of moisture content, K, and chl-(a+b). The correlation between them and spectral reflectance was in the visible region, the largest correlation coefficient was −0.77 (615nm), −0.72 (630nm), and −0.93 (572nm), respectively. These nutrients had a positive correlation with spectral reflectance in the NIR region where the largest correlation coefficient was 0.87 (770nm, 1057nm), 0.98 (770nm, 1057nm) and 0.57 (1057nm). The second group consisted of N, P, Ca, Mg, Zn, Mn, and Cu. The correlation between these nutrients and spectral reflectance was in the visible region. The largest correlation coefficient was 0.35 (680nm), 0.56 (660−680nm), 0.96 (630nm), 0.82 (630nm), 0.79 (662nm, 692nm), 0.85 (662nm), and 0.83 (667−687nm). These nutrients were negatively correlated with spectral reflectance in the NIR region. The largest correlation coefficient was −0.96

Table 3 Correlation coefficient among chl-(a+b), moisture and nutrients in leaf in the booting stage

r	W	chl	N	P	K	Ca	Mg	Zn	Mn	Cu	Fe
W	1.00										
chl	0.82*	1.00									
N	−0.35	−0.05	1.00								
P	−0.67	−0.29	0.84*	1.00							
K	0.95**	0.67	−0.57	−0.86*	1.00						
Ca	−0.89**	−0.85*	0.38	0.71	−0.89**	1.00					
Mg	−0.97**	−0.78*	0.50	0.81*	−0.99**	0.95**	1.00				
Zn	−0.86*	−0.61	0.62	0.92**	−0.95**	0.92**	0.95**	1.00			
Mn	−0.75	−0.76*	0.67	0.71	−0.79*	0.86**	0.84*	0.81*	1.00		
Cu	−0.74	−0.55	0.73	0.93**	−0.87*	0.89**	0.88**	0.97**	0.87*	1.00	
Fe	0.11	−0.30	−0.14	−0.06	0.11	0.33	0.02	0.13	0.25	0.26	1.00

$r_{(0.05,5)} = 0.7545$, $r_{(0.01,5)} = 0.8745$

(950nm), −0.96 (815− 830nm), −0.88 (775nm), 0.95 (750− 802nm), (850− 870nm), −0.98 (747− 892nm), −0.89 (972− 992nm), and −0.95 (742− 892nm, 997− 1092nm).

The last remaining element is Fe. The correlation coefficient between Fe content and spectral reflectance was 0.78 (440nm) in visible region. There was very little correlation with iron in any of the other visible or NIR regions.

In addition, when the correlation between nutrients and the spectral reflectances were large and positive, the curves were coincident. When the correlation between nutrients and the spectral reflectances were large and negative, the curves were symmetrical. This was most true for the correlation between nutrients in the inner leaves and spectral reflectances. Although 410nm, 550nm, 710nm, and 950nm were inflection points in most curves, correlation coefficients at these points were inconsitent. Future research plans are to discover the relationship between leaf spectral property, nutrients, and assimilative products, and the mechanisms that link them.

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不同钾素处理春玉米叶片营养元素含量变化及其光谱响应

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摘　要： 目的是研究不同钾营养水平春玉米典型生育期叶片的光谱响应，探索叶片内营养成分与叶片光谱反射率的相关性。方法是设置了不同梯度钾处理的盆栽试验，按玉米生育期进行光谱测定和取样分析。结果，通过对不同钾处理间玉米叶片养分含量的差异性分析表明，随着施钾的提高，叶片钾含量差异性达到显著水平。分析不同钾营养水平不同生育时期春玉米叶片光谱反射率与叶片钾含量的相关关系，并建立了喇叭口期利用叶片光谱反射率估测叶片钾含量的数学模型；以及分析了该处理下喇叭口期叶片内水分、叶绿素、氮、磷、钙、镁、锌、锰、铜、铁含量与叶片光谱反射率的相关性。结果表明：不同生育时期叶片钾含量与其光谱反射率的相关关系在光谱维方向存在明显差别，730-930nm 和 960-1100nm 两波段为春玉米喇叭口期评价钾营养状况的敏感波段，光谱变量 $R_{767}+R_{1057}$，$(R_{767}+R_{1057})/(\log R_{767}+\log R_{1057})$ 和 $(R_{767}-R_{1057})/(\log R_{767}-\log R_{1057})$ 均能很好的预测喇叭口期叶片钾含量；该时期叶片内不同成分与光谱反射率相关分析表明：550nm，710nm，950nm 三波段处是各个相关曲线的突变点；叶片内各成分间高度相关的，它们的光谱相关曲线趋势也极为一致或对称。

关键词： 春玉米；不同钾营养水平；营养元素；光谱反射率；相关性