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The Diagnosis of Components Information Distribution of Wheat Seedlings Based on the Hyperspectral Imaging

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Abstract: It is adopted mostly of the non-imaging spectrometer in current crop seedling monitoring, this method is greatly interfered by the soil background, makes it difficult to analyze the partial area nutritional status of the seedlings. In this study, we took advantage of merging the image with spectrum of the imaging spectrometer, to analyze the canopy, individuals, different size of leaves, characteristics of different regions of the wheat seedlings under the condition of salt stress, to diagnose the distribution of its chlorophyll composition information. We collected the imaging spectrum of 126 wheat samples in the wavelength range of 400 ~1000 nm, selected the average spectrum, exerted Correlation Analysis on the spectrum of wheat seedlings with the SPAD value, It could be seen that the biggest absolute value of the correlation coefficient was at 693 nm, which was considered as the characteristics wavelength of wheat seedlings. To establish the linear regression model using this wavelength and substituted 1the reflectance data of each point into the model, then we got the SPAD value of each point, to form the relative content distribution map of chlorophyll, whereby to diagnose the distribution of seedlings component. The results showed that: Hyper spectral imaging could reflect the reflectance differences of wheat seedlings under different salt stress treatments, through extracting the spectral reflectance curve leaves of single wheat seedlings in different parts of the different leaves and single leaf base, the midst of leaves and tip in the plant, from the results of filling map we could intuitively see the leaves' chlorophyll distribution in different parts. It indicated that hyper spectral imaging can characterize the seedlings situation of different plants, also could characterize the characteristics of different district of leaves. The results indicated that hyper spectral imaging were suitable for the non-invasive detection of chlorophyll content of wheat seeding and it has potential for precise diagnosing of the growing status of wheat seeding.

Keywords: Chlorophyll content, hyper spectral imaging, wheat seeding

INTRODUCTION

The growth status of seedling will affect the final biological yield, economic yield, nutritional quality and safety of the crops (Luo *et al*., 2009). Therefore, there has great significance for the precision agriculture to predict the growth momentum quality of wheat seedling effectively.

With the development of remote sensing technology, in particular the emergence and boosting of hyper spectral remote sensing technology, the spectral bands can be subdivided in a specific spectral region, has poured new vitality into the plant nutrition diagnosis. Scholars have carried out in-depth studies at components, structure, quality, nutrient, pest and disease stress and so on to wheat (Wang *et al*., 2004), rice (Huang *et al*., 2006), soybean (Song *et al*., 2005a), maize (Song *et al*., 2005b) and other crops using spectral analyzer of Field spec FR2500. Zhao *et al*. (2006) collected the multi-angle spectral information of winter wheat canopy using Field Spec-FR diagnosed the nutrient status of wheat at different levels; Wang *et al*. (2004) studied on rice canopy and the spectral information of leaves, constructed an inversion model of the pigment, the correlation coefficient was 0.7948. Wang *et al*. (2004) showed that on the relative water content of leaves with the characteristic water absorption peak near 1450 nm , a good linear positive correlation was existed, it could be used to quantitatively predict the water content and monitor the water shortage status of wheat. The Field Spec-FR used in these studies obtained the average spectrum targeting at measuring the light within the measurement region, it was non-imaging, mainly used for the diagnosis on late crops. In the early growth phases of crops, due to the lower ground coverage, the average spectrum collected was susceptible to the size of the clods, soil texture and dry weeds and it's difficult to obtain the spectrum of

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seedlings themselves accurately. Therefore, the non-imaging spectrometer is better for the diagnosis to groups of crops. As for the monitoring of seedlings which is more focused on the individual analysis, due to the large differences between individuals, it still lacks of effective detection method currently.

The hyper spectral imaging technology can obtain the spectrum of each pixel of the image, can analyze the spectrum of each local area, can also accurately extract images of seedlings under different bands and to select the image under the feature band, to execute image processing. The advantage of merging the image with spectrum of hyper spectral imaging technology makes it a great potential in the modality detection, component diagnostics and a great predominance for the study of individual differences, it was mainly used in aviation, satellite remote sensing initially, with the lowering of costs, it is possible for the near-Earth applications. Some scholars carried out in-depth studies at fruit internal quality, meat quality classification , detecting plant diseases and insect-damaged wheat kernels using the hyper spectral imaging.

In this study, we took advantage of merging the image with spectrum of hyper spectral imaging technology, to obtain the hyper spectral images of wheat seedlings under different levels of salt stress using of imaging spectrometer, analyzed the characteristics of wheat canopy, individual, different size of leaves and different regions, diagnosed the composition information distribution of its chlorophyll content.

MATERIALS AND METHODS

Design of the experiment: The wheat varieties selected in this study were of Chinese Spring, Zhou Yuan 9369, Changwu 134, the test was executed indoors. After the seeds were disinfected with 3% sodium hypochlorite solution for 15 min, soaked in distilled water for 24 h, selected the consistent sprouting seeds to place in the germination box which lined with a layer of filter paper, executed salt stress by adding NaCl to seedlings, to inhibit the growth of seedlings, adding water as a control for normal growth of seedlings, replaced the nutrient solution and filter paper daily. The culture conditions were of light incubator, the temperature cycle was 20/25℃, photoperiod was 12h, the relative humidity was 60%. Each box had 12 holes well, each hole contained 2 wheat plants, each species occupied four holes. Waited to tiller of wheat, the seedling stage usually appeared four leaves, then the spectral images and chlorophyll values of seedling canopies, single plant and leaves was collected.

Hyperspectral imaging system: The Pushbroom Imaging Spectrometer (PIS) adopted in this study was jointly developed by the Beijing Research Center of Agricultural Information and University of Science and Technology of China. To employ the characteristic of merging the image with spectrum of this instrument, could we closely explore the individual crops, organs and spectral information of different organ components, to execute qualitative and quantitative studies to the agriculture crops from different scales. The system consists of imaging spectrometer, motor, rail, adjustable halogen lights and external laptop. Before applying of the spectrometer, we authorized the professional spectrometer detection organization in China to calibrate and test, to determine the wavelength position, the spectral response function, radiation accuracy, spatial positioning of each channel in the spectrometer, in the future it will be adjusted the calibration parameters and calibrate on a regular basis corresponding to the performance changes of the instrument. (The specific performance parameters are in Table 1).

The methods of leaf spectral data acquisition and processing: This experiment was carried out in Beijing Research Center for Agri-food Testing and Farmland Monitoring, in 2010. The winter wheat varieties were Zhou Yuan 9369, Chinese Spring, Changwu 134. The vertical height of sample to spectrometer lens was 66 cm, the motor speed was 85 mm/s, after the various parameters of the acquisition software were set, turned on the halogen to beam, to collect the spectral image on the canopy, plant, leaf of the wheat seedlings with PIS, to collect canopy spectrum in 10 groups for wheat seedlings and selected 6 wheat seedlings from each group, 3 single leaf with differences in representation were chosen from per plant. To collect the data of hyperspectral imaging of the samples leaves with PIS imaging spectrometer, the total measurement data was 7 groups, the each single-leaf test sample had 18 single leaves, a total of 126 samples were obtained.

The raw data collected by imaging spectrometer was in BMP format, it might to be spliced into image of BIL format before using. Process was as below:

- Splice BMP format images into a whole lot of BIL format image using the Matlab program edited by ourselves
- Extract spectral from the original spectral images using remote sensing image processing software EVNI 4.4 (the Environment for Visualizing Images). The spectrum of the leaf base, middle leaf

Table 1: The key performance parameters of spectrometer

Spectrum range	400-1000 nm	Sampling interval	0.7 nm
Spectrum resolution	2 nm	Pixel dimension	7.4 μm×7.4 μm
Spatial resolution	0.5 mm	FOV	24°
Image resolution	1400 (Spatial)×1024 (Spectrum)		

and tip of leaves were extracted respectively and averaged, as the original spectral values of the target leaves.

Method of reflectivity calculation: executing conversion on the collected hyperspectral reflectance values of canopy, single plant, single leaf in the extracted wheat seedlings with the spectral reflectance of the white board simultaneous determined, achieved the target spectral reflectivity by the following formula:

Reflectance:

$$R_{target} = \frac{\mathrm{Rad}_{\mathrm{target}}}{\mathrm{Rad}_{whiteboard}} \times \mathrm{Ref}_{\mathrm{whiteboard}} \times 100\% \quad (1)$$

where, R_{target} was the target spectral reflectivity data obtained through the spectral reflectivity of white board in the formula; Rad_{target} was the target radiance value measured by the spectrometer; $\mathrm{Rad}_{whiteboard}$ was the white board radiance value measured by the spectrometer; $\mathrm{Ref}_{whiteboard}$ was known as reflectance values of whiteboard.

Determination of chlorophyll content: Selected leaves from tip to the 3rd count backwards of per plant, to measure the relative content of chlorophyll or "green level" of wheat seedlings by the chlorophyll content analyzer of SPAD-502. To measure the chlorophyll relative value of a single leaf at the site corresponding to the single wheat seedlings which was measured spectrum, tested 3 times in leaf base, middle leaf and leaf tip respectively and averaged which to calculate SPAD value.

RESULTS AND DISCUSSION

The comparative analysis of single plant wheat seedlings with imaging spectrum of different scales of leaves: Extracted the images under R, G, B primary colors wavelength (680, 550, 450 nm) from the ENVI software to reconstruct and obtain RGB images, the RGB images of wheat canopies, single plants, single leaves were shown, respectively in Fig. 1, 2 and 3, The color could be analyzed of the seedling according to the extracted RGB images. The yellow leaves could be clearly seen in Fig. 2 and 3, which suggested that these leaves were affected by the salt stress, lack of nutrition.

Fig. 1: The RGB image of wheat canopy

Fig. 2: The RGB image of wheat seedling single plant

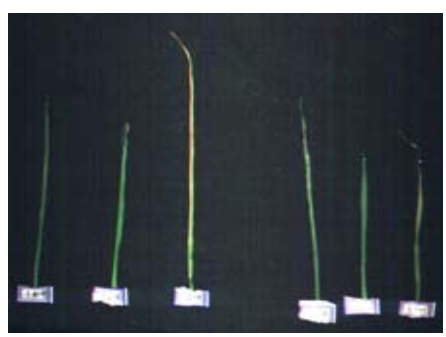

Fig. 3: The RGB image of wheat leave

The qualitative analysis on the nutritional status of wheat seedlings could be directly executed by Hyper spectral imaging technology.

The spectral reflectance of a single plant and leaves in the wheat seedlings were extracted respectively at wavelength range of 400~1000 nm for analysis (Fig. 4). It could be seen from the figure, the reflectance absorption peak was at the leaf spectrum of 550 nm, the reflectance absorption valley was at 680 nm, the above

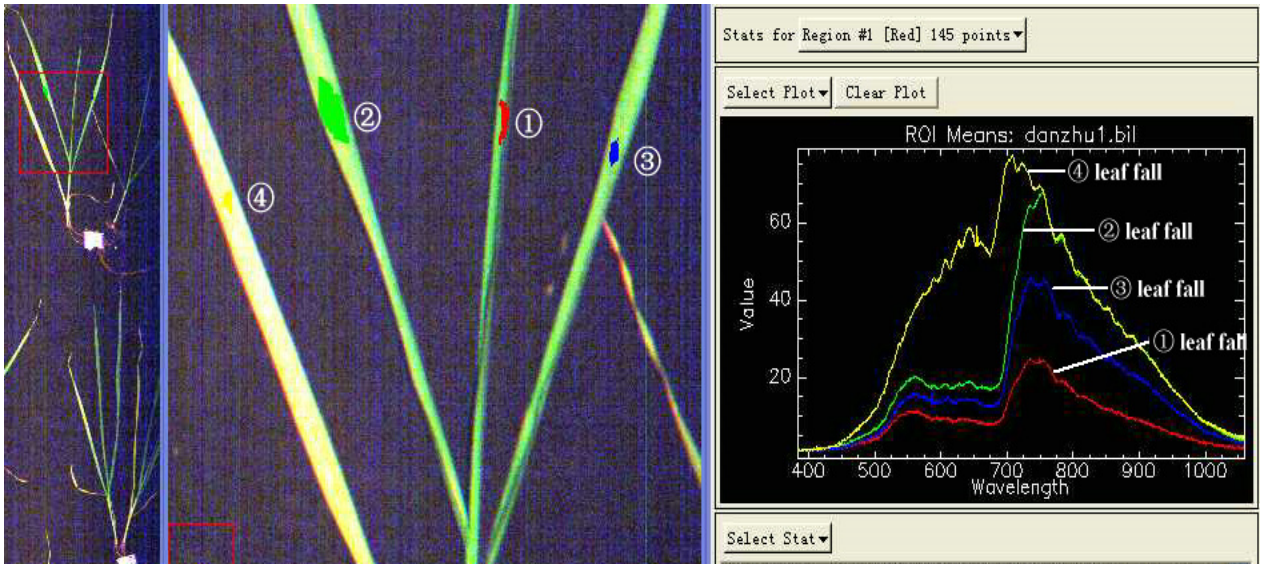

Fig. 4: The spectral reflectance curve of different leaf in single plant wheat seedlings

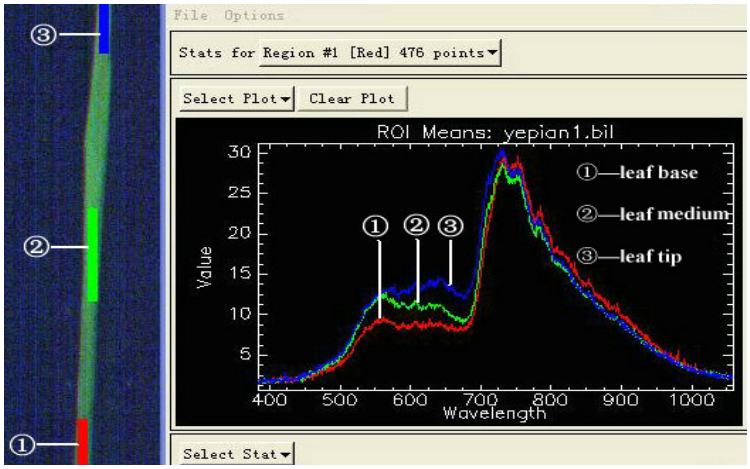

Fig. 5: He spectral reflectance curve of different parts in wheat leaves

two wavelengths were the typical spectral variation features of vegetation [13]. Executed contrastive analysis on the hyper spectral reflectance extracted from the 1st, 2nd, 3rd, 4th count backwards leaf of single wheat seedlings showed that: there was difference of spectral reflectance value in different single wheat seedlings, spectrum of the 4th count backwards leaf was higher than the other three leaves, this might be due to the uneven nutrient transport in the seedlings growth process, the 4th count backwards leaf appeared yellow, showed high reflectivity; For the different parts in single leaf (Fig. 5), extracted the spectral reflectance curve of from middle leaves, leaf base, tip parts, it was different in spectral reflectance of the three point, among which the reflectivity of tip was higher than the other two places. To analyze these results, followed the transfer rules of nutrients from the roots to the tip, the leaf tip turned yellow aging first, when the wheat seedlings subjected to salt stress, so there were relatively large range of variation for the chlorophyll content and spectral reflectance values. General after the leaves suffered lacking of nutrition, the tip area feel the change first, so the reflectivity of the tip was high. The wheat reflectance measured in this study was 0.20, there existed differences with the hyper spectral reflectance of achieved in the leaves of the late wheat under the condition of high stress, the reflectivity they measured was 0.25, analyzed the

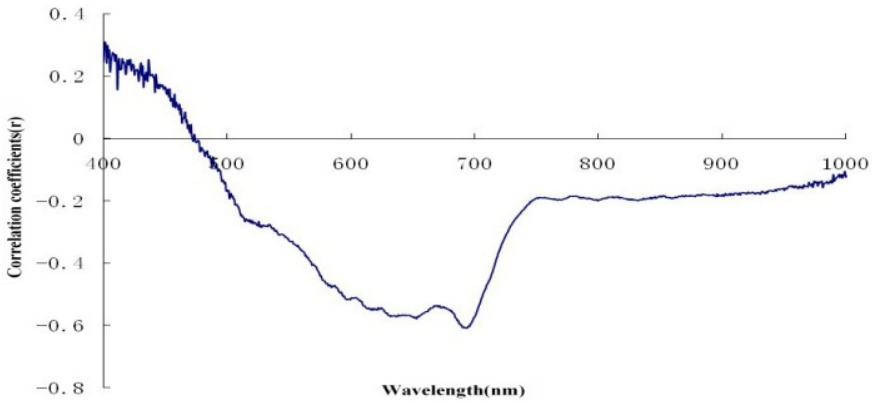

Fig. 6: The correlation between the spectral reflectance of wheat seedlings and chlorophyll SPAD values

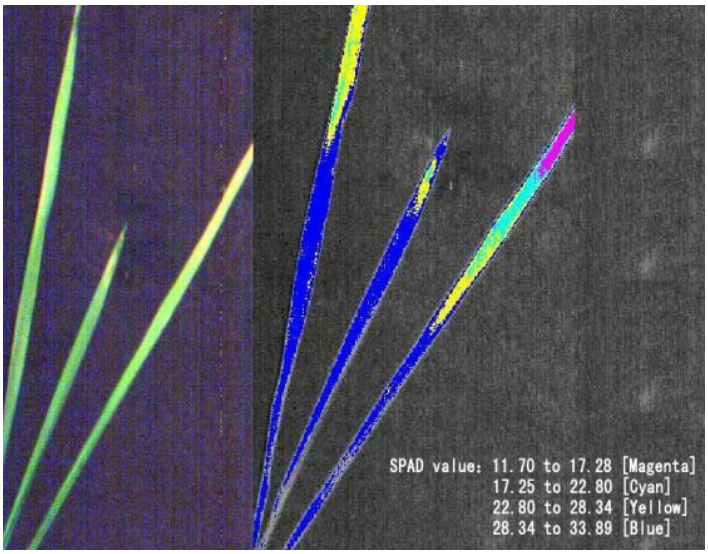

Fig. 7: The forecasting map of wheat seedlings filling map

reason for the difference might be due to the growing enough and good nutrition of the wheat leaves in the late stage, so the spectral reflectance of the late wheat leaves was higher than that of the wheat seedlings leaves.

Selected the diagnostic wavelengths of wheat seedlings, to establish the correlation analysis: Extracted the average spectrum of wheat samples in the wavelength range of 400~1000 nm, to exert correlation Analysis with the SPAD value, the correlation coefficient was shown in Fig. 6. It could be seen that the leaves of wheat seedlings was significant positive correlate with the reflectance of chlorophyll SPAD values in the visible light between 400~471 nm, while it was significant negative correlated between the wavelength of 471~1000 nm. When the wavelength was between 690 nm~697 nm, the value of correlation coefficient was at trough (r<-0.60), it was lowest at 693 nm. Without respected its' negative or positive, selected the wavelength that the maximum point of the absolute value of the correlation as the largest coefficient, that was to say 693 nm was of the characteristic wavelength of the wheat seedlings correlation. It was possible to take advantage of this characteristic wavelength to carry further quantitative analysis on the chlorophyll SPAD values of wheat seedlings with the corresponding imaging spectrum, to establish an appropriate regression equation for monitoring chlorophyll component of wheat seedling.

Characteristic wavelengths chosen to establish regression model for prediction of filling map: In this study, 693 nm was taken as the characteristic wavelength, the samples were of 126, to establish the linear regression model with the chlorophyll SPAD

value of wheat seedlings, the correlation coefficient R was 0.607, The modeling result was shown as Eq. (2):

$$SPAD = -0.994186592 * x1 + 38.72313056 \quad (2)$$

To substitute the reflectance data of each point into the model, the chlorophyll content of each point could be gained and chlorophyll content distribution was formed (Fig. 7). From the results of filling map, the distribution of chlorophyll could be visually seen in different parts of the leaves, the tip part turned yellow for lacking of nutrition, the yellow part of the far right blade tip was shown as pink in the results of filling map in Fig. 7, it suggested that this was obvious different with the green part of the normal leaves and it would be distinguished with different colors corresponding to the different changes of chlorophyll content when filling map, refinement of diagnosis could be carried out by predicting of filling map to achieve the distribution of the components information in local areas of wheat seedling leaves, which was the characteristics of the imaging spectroscopic technology superior to the conventional non-imaging spectroscopic technology. The current monitoring seedling mostly applied of non-imaging Field Spec-FR, its shortcomings primarily were:

- It was non-imaging, the spectral information collected of the wheat canopy was mixed, resulting in large interference to the growing wheat seedlings by the soil background
- It provided "point"-like data, made it difficult to analyze the nutritional status of the local area to the seedlings
- Unable to detect the spectral information at different levels of wheat accurately.

It was comprehensive utilizing the advantages of merging the image with spectrum of hyper spectral imaging in Fig. 7, obtained the chlorophyll distribution, qualitative and location analysis on the wheat seedlings could be achieved on the basis, can accurately detect components of wheat seedling nutrition information, to achieve predicting the distribution of chlorophyll components of the visual display of information, wheat seedling nutrition components information could be accurately detected, to achieve the visual display of forecasting the distribution information of chlorophyll components.

CONCLUSION

In this study we took advantage of merging the image with spectrum of the imaging spectrometer, achieved spectral imaging maps of the canopies, single plants, leaves of the growing wheat seedlings under salt stress condition, through the analysis to the chlorophyll values of different layers, it was found that there was significant difference in the chlorophyll value of leaves between different layers. It could be clearly distinguished yellow leaves from growing withered of wheat seedlings from the figures, suggested that hyper spectral imaging could achieve for the visual qualitative analysis of wheat seedlings. By extracting the spectral reflectance curve of the different leaves and leaf base, leaves, tip of single leaf of wheat seedlings, indicated that there was significantly different of the spectral reflectance in wheat seedlings under salt stress. The correlation analysis was carried out on the average spectrum of wheat seedling leaves extracted between wavelength range of 400~1000 nm with the chlorophyll SPAD values, found that the largest absolute value of correlation coefficient was at the wavelength of 693 nm, was characteristics wavelength of chlorophyll of wheat seedling. The linear regression model was established between the chlorophyll SPAD value and the characteristics wavelength using 126 samples and substituted data of the reflectivity of each point into the model and obtained the chlorophyll content of each point, to form the chlorophyll content distribution, from the filling map could visually see the results of the distribution of chlorophyll in different parts of leaves, thereby achieved the visual diagnosis of nutritional status distribution of local area in wheat seedlings.

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