

Change characteristics of chlorophyll content and high spectrum in soybean leaves under the stress of CO₂ leakage

Ruijuan Hao^{1,2}, Zhoufeng Wang^{1,2}, Wenke Wang^{1,2*}, Yaqian Zhao^{1,2}

¹ Chang'an University, Environmental Science and Engineering College, Shaanxi Xi'an, 710054, China

² Key Laboratories for Underground Hydrology and Ecological Efficiency of the Education Ministry of China, Shaanxi Xi'an, 710054, China

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The carbon capture and storage (CCS) may have the problem of leakage and ecosystem equilibrium may be changed due to the affected soil biological system and plants after CO₂ leaking into the soil. By measuring the hyperspectral reflectance and chlorophyll content in soybean leaves under the stress of CO₂ leakage as well as the continuum removal disposal and derivative spectrum calculation for hyperspectral data within the wavelength range of 400~800nm, 10 spectral feature parameters of absorption valley position, normalized reflectance at the absorption valley position, absorption depth, green peak position, normalized reflectance at the green peak position, red edge position, normalized reflectance at the red edge position, red shoulder position, absorption width and spectrum symmetry could be achieved. The correlation analysis of high spectrum and change characteristics of chlorophyll content in soybean leaves based on analysis of physical significance for above parameters is thus conducted. The result has shown that, under the stress of CO₂ leakage, the chlorophyll content change, absorption valley position, normalized reflectance at the green peak and red edge position are closely related and in this way, the chlorophyll content equation: $Y = -19575 + 11.9X_1 - 45.1X_2 + 17.6X_3$ is obtained. The result of study in this paper has an important practical significance and application value for ground surface eco-environment monitoring and evaluation of CCS projects and remote sensing monitoring of CO₂ leakage points.

Key Words: Hyperspectral reflectance, Chlorophyll content, Stress of CO₂ leakage.

INTRODUCTION

Intergovernmental Panel on Climate Change (IPCC) has pointed out in 2014 Report that, the global surface temperature was increased an average of 0.85 °C from 1880 to 2012. It was mainly caused by greenhouse gases, to be specific, the emission of CO₂ [1]. The carbon capture and storage (CCS) can capture, liquefy and geologically sequester the CO₂, which helps reduce the emission of CO₂. For this reason, it is a novel initiative to cope with the global climate changes [2]. CCS engineering projects operate well and no CO₂ leakage examples are reported, but leakages may also occur in the storage period. The ecosystem equilibrium may be changed due to affected soil biological system and plants after CO₂ leaking into the soil [3-5]. How to formulate a set of effective means to rapidly detect the CO₂ leakage points in the CCS period is the key link for geological storage of CO₂. The surface monitoring can only be applied for small areas, while in large areas, lots of manpower and equipment investment

response of plants. Based on this, leakage points of CO₂ may be found out by monitoring spectral characteristics of stressed plants by the remote sensing monitoring technique [6].

The chlorophyll is a basic composition of plant leaves, whose content is an important condition for exchange of substance between plants and the outside world as well as a significant index for plant nutritional stress and growing development state [7-9]. The growth of plants could be monitored and stress degree of plants could be estimated upon monitoring for chlorophyll content changes in the Soybean leaves. The conventional chlorophyll measurement methods would take a long time period and cause damages to the vegetation. The measuring accuracy may be affected by the chlorophyll content changes due to the lost part of chlorophyll during the transportation and sample preparation from field to laboratory [10]. As a non-invasive chlorophyll measurement method, high spectrometer could measure the chlorophyll content by measuring the reflectance, transmittance and absorptive of green plant leaves [11]. Therefore, it decides the unique effect for high spectrum technology in the measurement of chlorophyll content. The spectral characteristics of ground vegetation and bio-physical parameters present a significant correlation. The study result of Yunhao

To whom all correspondence should be sent:
E-mail: wenkew@chd.edu.cn

are required. The plants are often covered in geological storage areas of CO₂, so leakage may lead to the increased CO₂ concentration and stress

CHEN, et al [12] found that, the stress of CO₂ leakage may obviously reduce the beet chlorophyll content and leaf water content, wherein the leaf reflectance is reduced at 550nm and increased at 680nm. The study result of Jinbao JIANG, et al [13] found that, if the volume fraction of CO₂ in the soil is equal or greater than 15%, Area (510~590 nm) index can better recognize the stressed soybeans of higher separation ability and stability. However, if the volume fraction of CO₂ in the soil is smaller than 15%, Area (510 ~ 590 nm) index cannot exactly identify the stressed soybeans during the whole growth period.

The reports for change laws of chlorophyll content by hyperspectral characteristic parameters under the stress of CO₂ leakage are based on the artificially controlled CO₂ release concentration and there's no report on change laws of chlorophyll content by hyperspectral characteristics under the stress of natural CO₂ leakage. There are abundant data materials available by high spectrum remote sensing technique. There's no unified definition to determine which parameter could have a more significant reflection on change characteristics of vegetation stressed by the CO₂ leakage. Hence, this paper used the soybeans grown under the stress of natural CO₂ leakage as experimental materials, analyzed the differences in reflectance spectrums of soybean leaves by measuring multi hyperspectral parameters and chlorophyll contents in different leakage conditions, delved into hyperspectral characteristic parameters under the stress of natural CO₂ leakage as well as selected and calculated the built-up model of spectral characteristic parameters that could effectively reflect the stress influence of soybeans.

SELECTION OF TEST AREA AND TESTING METHOD

Selection of test area

The test area is located in First-Terrace Trailing Edge of Qijiachuan River Valley, 0.5km away from Sanhe Town, Ping'an County, Xining City, Qinghai Province, wherein an artesian hot well of CO₂ gas-water mixing high pressure is drilled out. The well is located at 36°25'29"E and 101°56'28"N, whose depth is 192.08m, aquifer thickness of CO₂ gas-water mixing is 35.09m, artesian discharge at the well mouth is 156.82m³/d and water temperature is 17 °C.

Testing method

Measurement on effect of CO₂ leakage stress in test area. This test area is an area dividing the

vegetation effects in the shape of half round, which is centered by the artesian hot well of CO₂ gas-water mixing high pressure, in where soybean plants were randomly selected in testing areas for measurement of spectrum and chlorophyll content. A CO₂ testing probe was installed in each testing area to measure the CO₂ leakage concentration and hence to determine the heavy stress area, medium stress area and mild stress area. At the same time, the local soybean plants free from CO₂ are selected to act as the control blank (CK).

Measurement on simple-leaf spectrum. This paper measured the soybean leaf spectrum by blade clip allocated by ASD Field Spec 4 from 10AM to 2PM on 10 August 2015. The measurement was conducted under the condition of stable light and cloudless sky with the wind power less than level 3. In order to measure the influence on spectrum measurements by rolled leaves, the measurement on each leaf for 4 times and each time for 90°. The correction using the standard white plate was conducted for once on each leaf. For measurement, 10 leaves of same growth conditions were selected in each test area and the mean was selected as the spectral value of leaves in this testing area.

Measurement on chlorophyll content. The measurement on chlorophyll content in soybean leaf by CCM-300 chlorophyll measurement instrument at the moment the spectroscopic data was collected. This paper selected different leaves of upper, middle and lower vertical layers of soybean plants on each collection point to avoid the venation, wherein 10 soybean leaves were selected from each layer and totally 30 soybean leaves in each test area. The mean value was thus acted as the data of chlorophyll content in soybean leaf.

Pre-processing of spectral data

Five-spot smoothing method. The smoothing process of spectral data could effectively remove the interference of high-frequency noise, wherein the denoising capacity may be enhanced due to the increased smoothing points. But if the selected smoothing points were too large, they would remove some useful signals as well and thus lead to the signal distortion. This paper made the smoothing preprocessing of spectral data by using the five-spot smoothing method and after thus processing, the spectral outline was more clear [14].

Continuum removal. The reflectance is not only affected by the biophysical and biochemical parameters of vegetation, but also is sensitive to light conditions when sampling. The absorbed electromagnetic waves by the vegetation mainly occur within the scope of visible spectrum. For this

ground, continuum removal was used for normalized processing of reflectance spectrum within the waveband of 400-800nm. In this way, weakening the influence of light conditions when collecting the spectrum could achieve the comparability between data, whose values reflect the differences in amount of information carried in the absorption characteristic waveband period by spectrum with different exchange ways. Due to these reasons, the continuum removal is a spectrum analysis method effectively enhancing the interested absorption characteristics [15].

Derivative spectrum. The spectrum position represented by maximum value of first-order differential in the reflectance spectrum within the vegetation red edge waveband is called as “red edge” [16]. The calculation way for the first-order derivative of reflectance spectrum is presented as follows:

$$D_{\lambda_i} = \frac{R(\lambda_{i+1}) - R(\lambda_{i-1})}{\lambda_{i+1} - \lambda_{i-1}}$$

In the equation, D_{λ_i} is the first-order derivative spectrum of wave length λ_i and $R(\lambda_i)$ is the spectral reflectance of wave length λ_i , wherein λ_i is the wave length of each waveband.

RESULTS AND ANALYSIS

Biology characteristic of soybean under different CO₂ leakage stressed level

If CO₂ of certain concentration was leaked into the vadose zone, replacement reaction between it and oxygen in the soil may occur and thus lead to the soil acidification. Hence, the photosynthesis and respiration of plants may be limited and biology characteristics of vegetation may be changed [17-19]. This paper observed and recorded the leaf shape, color, plant height, stem growth situations and biology characteristics of legume under different CO₂ leakage stressed level, and the results are presented in Table 1.

Table 1. Biology characteristics of soybean under the stress of CO₂ leakage

Biology Characteristics	Heavy Stress	Medium Stress	Mild Stress	Control Blank
Leaf Shape	Mostly Curved	Partly Curved	Slightly Curved	No curve
Leaf Color	Yellow Green	Yellow Green	Green	Green
Plant Height	140cm	145cm	152cm	159cm
Plant Stem	Partly Lodged	Slightly Lodged	No Lodge	No Lodge
Legume	Mostly Curved	Partly Curved	Slightly Curved	No curve
CO ₂ Concentration	30-40%	20-30%	10-20%	0.3%

From Table 1, the soybean leaf and legume under different CO₂ leakage stressed level are curved in varying degrees and when the CO₂ stressed level reaches over 20%, the leaf color may be affected. Specifically, those plants may present a color of yellow green than healthy leaves, stems may lodge at different levels and the plants may present the feature of small size. It can thus be seen that, the CO₂ concentration in normal soil is relatively low; if CO₂ concentration is increased to 10%, it may cause a significant adverse effect on the growth of plants; if CO₂ concentration is increased to 20%, it may obviously impact the biology characteristics of plants; if CO₂ of high concentration may exist on land surface for a long time, which plants are hard to live.

Spectral characteristics analysis

Absorption valley position and absorption depth. The wavelength corresponded by the lowest point on the reflection curve of soybean leaf was acted as the absorption valley position and the absorption extent of that wavelength point was calculated as the absorption depth to reflect change

features of absorption valley position on the leaf spectrum under different CO₂ leakage stressed level.

Figure 1 is the reflectance spectrum of disposed soybean leaf by five-spot smoothing method and continuum removal method. The photosynthetic pigment in palisade tissue may strongly absorb the blue and red light and relatively reflect green light, the soybean spectrum curve may thus lead to an absorption valley (red light of about 670nm) and a reflection peak (green light of about 550nm) in the visible spectrum (400-800nm).

The largest absorption valley position among all kinds of CO₂ leakage stress and control blanks is located at approximately 670nm with the biggest variability rate of 5nm, showing that the position of absorbed valley bottom is determined by characteristics of the absorbed substance (refer to Table 2). The green peak height of soybean leaf stressed by CO₂ leakage was obviously greater than that in the control blank and the depth of absorbed valley bottom was shallower than that in control blank. As the CO₂ leakage stress increases, the absorption depth presents the reduction trend (refer to Figure 1). It is mainly caused by the

reduced absorption capacity when the soybean leaves were stressed by the CO₂ leakage. It can be seen that, the absorption depth of the absorbed valley bottom is determined by the absorption capacity of visible light by the soybean leaves.

Green peak position and red edge position.

From Table 2, the difference between green peak positions in CO₂ leakage and control blank is not significant. The green peak position in the heavy and medium stress area is at approximately 554nm, while the green peak position in the mild stress area and control blank is at approximately 552nm. It has indicated that the green peak position has no determined numerical relationship between the CO₂ leakage stress extent (refer to Table 2). However, as CO₂ leakage stress enhances, the chlorophyll content in soybean leaves would

present an obvious reduction trend and the reflectance of soybean leaves in different CO₂ leakage areas may increase due to the reduced chlorophyll content in areas centered by 550 nm (refer to Figure 1). It has indicated that the CO₂ leakage stress may exert some impacts on the green peak characteristics by exerting the impact on the chlorophyll content.

As the most conspicuous symbol of plant spectrum, the red edge is caused by the strong reflection, which is formed by strong absorption of chlorophyll in red waveband and multiple scattering inside of leaf in the near-infrared waveband of plants [20]. This paper showed that, when the leaves were stressed by the CO₂ leakage, the red edge wavelength may have the following

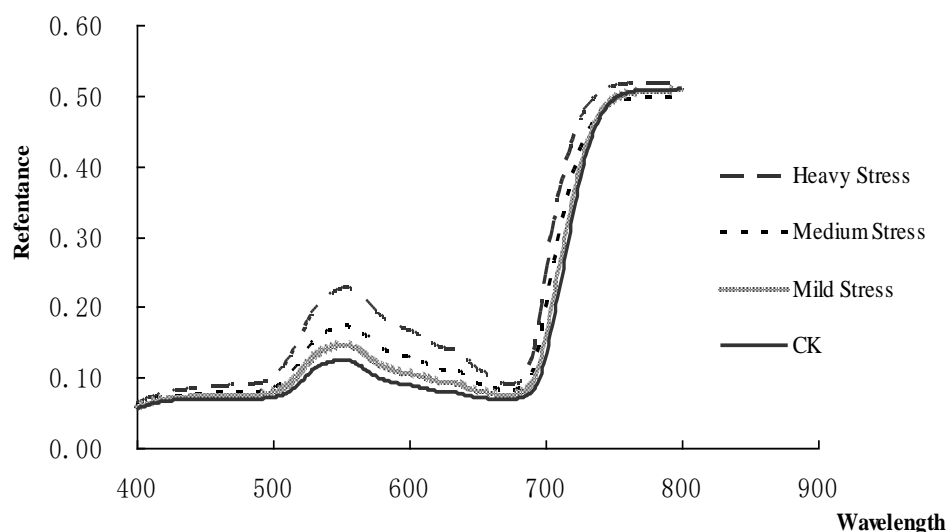


Fig. 1. The leaf reflectance curve of soybean after the pretreatment.

Table 2. Spectral characteristics of soybean under the stress of CO₂ leakage and control blank.

Spectral Characteristics	Heavy Stress Area	Medium Stress Area	Mild Stress Area	Control Blank
Maximum Absorption Wavelength (nm)	673	672	671	668
Reflectance at Maximum Absorption Wavelength	0.0898	0.0808	0.0738	0.0681
Maximum Absorption Depth for Spectral Band	0.9102	0.9192	0.9262	0.9319
Green peak Wavelength (nm)	554	554	552	552
Reflectance at Green peak Wavelength	0.2266	0.1734	0.1456	0.1249
Red Edge Wavelength (nm)	699	702	707	715
Reflectance at Red Edge Wavelength	0.0137	0.0115	0.103	0.0107
Red Shoulder Wavelength (nm)	774	785	791	799
Absorption Width (nm)	220	231	239	247
Degree of Symmetry	0.3254	0.2320	0.2262	0.1898
Chlorophyll Content	720.07	763.27	840.47	946.23

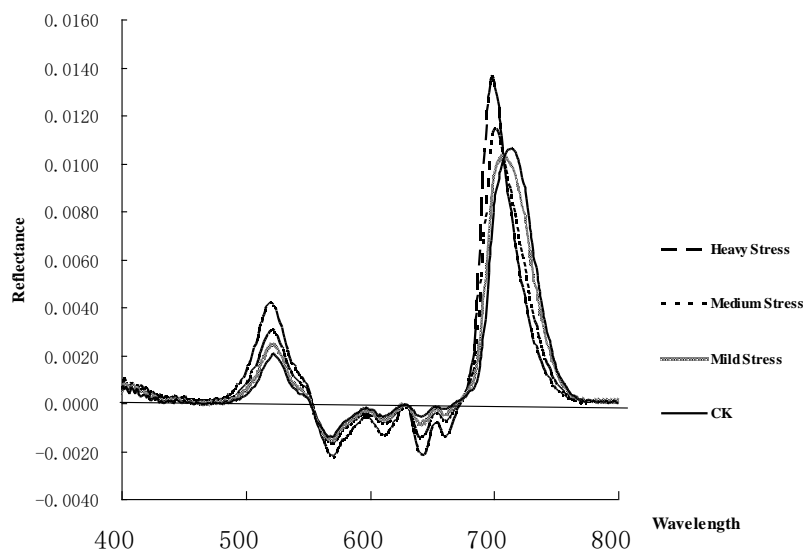


Fig. 2. Derivative spectrum of soybean leaf under the stress of CO₂ leakage.

relationship: heavy stress area (699nm) <medium stress area (702nm) <mild stress area (707nm) <CK (715nm). That is to say, the red edge of soybean leaf may move to the short wave direction as the CO₂ leakage stress extent enhances, i.e. blue-shifted phenomenon (refer to Table 2). It thus appears that the red edge position is a good index to reflect the CO₂ leakage stress.

Absorption width and red shoulder position.

Absorption width refers to the wavelength difference between red shoulder position and green peak position. This study has demonstrated that, the variation tendency for absorption width and stress level of soybean leaf spectrum curve stressed by the CO₂ leakage may be opposite. The larger the stress level is, the smaller the absorption width would be (refer to Table 2).

Red shoulder position refers to the wavelength position where the maximum reflectance in visible light waveband located. This study has demonstrated that, the red shoulder position has something to do with the effect of CO₂ leakage stress, wherein the maximum reflectance wavelength at the red shoulder position in the unstressed areas is the largest. It is unanimous to the red edge position and so does the change law of absorption width (refer to Table 2).

Degree of symmetry. The degree of symmetry refers to the ratio between left side area of absorption and the absorption area, reflecting the symmetry of spectral reflectance characteristics. This study demonstrated that, as the CO₂ leakage stress level increases, the spectral curve may present an increased trend in areas in the direction of short wave (refer to Table 2).

Measurement and analysis for chlorophyll content

Variance about the chlorophyll content.

This paper made the single factor variance analysis for chlorophyll contents of soybean with control blank and under different measured CO₂ stresses, and under the condition of variance analysis results significantly.(as shown in Table 3). Based on this, the least significant difference (LSD) method was adopted to make multiple comparisons in stress and control blanks and thus to determine the difference of chlorophyll content in all kinds of treatments. The results are presented as below:

Table 3. Variance about the change of chlorophyll content of soybean under CO₂ stress.

Source of variation	SS	S ²	F
Among treatment	3 920822.42	306940.81	44.11**
Within treatment	16 807174.57	6958.40	
Total variation	119 1727996.99		

*) F (3,116)x0.05=2.69; F (3,116)x0.01=3.96

Table 4. The results of multiple comparisons about the change of chlorophyll content of soybean under CO₂ stress (LSD).

Treatment	Chlorophyll Content	0.05	0.01
CK	946.23	a	A
Mild Stress	840.47	b	B
Medium Stress	763.27	c	C
Heavy Stress	720.07	d	C

From factor variance analysis and multiple comparison results for changes of chlorophyll content, the chlorophyll content in the control blank is significantly greater than other treatments stressed by the CO₂ stress to an extreme extent; a significant

difference, but not an extremely significant difference, exists between the heavy stress and medium stress treatment, while there's a significant difference to an extreme extent between the heavy stress and mild stress (refer to Table 4). It follows that, if the soybean was suffered from the effect of CO₂ stress, its chlorophyll content may be reduced as the stress level increases.

Stepwise multiple regression equation for chlorophyll content. By taking the above 10 spectrum parameters as the independent variable and chlorophyll content as the dependent variable, the following relationship is obtained by the stepwise multiple regression:

$$Y = -19575 + 11.9X_1 - 45.1X_2 + 17.6X_3$$

Wherein, X₁ is the absorption valley bottom position, X₂ is the normalized reflectance at the green peak position, X₃ is the red edge wavelength and coefficient of determination is R²=0.8942. It has shown that, the absorption valley position and normalized reflection at green peak of soybean leaf obtained by measuring the soybean leaf spectral curve as well as the red edge wavelength achieved by the derivative function could invert the relation between the hyperspectral curve and chlorophyll content changes stressed by the CO₂ stress. Besides, chlorophyll content respectively has a negative correlation with the normalized reflection at green peak and positive correlation with red edge wavelength. The normalized reflection at absorption valley bottom, absorption depth, green peak wavelength, normalized reflection at red edge, red shoulder wavelength, absorption width and degree of symmetry cannot better reflect the changing relationship between the soybean leaf spectral curve and chlorophyll content stressed by the CO₂ stress. For these grounds, these parameters are not suitable to be selected to invert the law of change for the stressed chlorophyll content.

DISCUSSIONS

The absorption, reflection and scattering effect on light by plants constitute characteristic spectrum. The reflection low valley in the visible band (blue light and red light at 0.45 and 0.67μm) is mainly caused by strong absorption of photosynthetic pigments inside of palisade tissue. The "reflection plateau" formed at the near-infrared band (0.7-1.3μm) is caused by multiple reflections and scatters of "water-air interface" in the internal structure of plant leaves (spongy tissue). There are 3 obvious absorption valleys in the middle-infrared band greater than 1.3μm (located at 1.45, 1.94 and 2.7 μm, respectively), which is mainly caused by the

strong absorption of liquid water in the leaves [21]. The "blue edge" (transited from blue to green) in the visible band, green peak, "yellow edge" (transited from green to red), red light low valley and near-infrared "red edge" transited from the red light are important indicating bands to describe the leaf pigment situation and physical condition. This paper found that, affected by CO₂ of high concentration, the visible band and near-infrared band of plants both present a relatively significant difference, having a better correlation with CO₂ concentration. It also demonstrated that the information materials stressed by CO₂ leakage by measuring the plant spectral data could be obtained under the field in-situ conditions.

Some studies on response problems of aboveground vegetation caused by leakage of CO₂ geologic sequestration at home and abroad have been conducted, but most were made under the condition of artificially controlled CO₂ concentration, which has some differences with the natural CO₂ leakage method and law. This paper employed the natural CO₂ leakage conditions to analyze physiological responses and hyperspectral characteristics of plants, whose results may better reflect the actual CO₂ leakage situation. Upon study on spectral change characteristics of the aboveground vegetation stressed by CO₂ leakage, Yunhao CHEN, et al designed ratio R550/R680 to recognize the beet stressed by CO₂ leakage. The study made by Jinbao JIANG, et al was designated to adopt the area vegetation index Area (510~590nm) to recognize the soybean stressed by CO₂ leakage. But among relatively many parameters reflecting spectral characteristics changes of vegetation, which parameter(s) would better reflect the spectral characteristics stressed by CO₂ leakage? There's no unified definition to answer such question. Then spectral feature parameters were selected by this paper to conduct the analysis. By selecting ten main spectral parameters and making analysis for these parameters, this paper demonstrated that, the red edge position, absorption width, red should position, degree of symmetry and other important element have a significant difference under different CO₂ concentrations, respond to the varying environment at all kinds of levels and the indicated technology indexes would also have certain difference. Hence, the single-factor indication and discrimination method may undervalue or overvalue the reaction to the external environment by plants. This paper used the stepwise regression to find out 3 characteristic parameters closely related to the chlorophyll content of soybean leaf, preliminarily established the regression equation integrating the responsive

technical indexes and thus obtained a more reliable result in theory. Featured in the low cost, large monitoring area and instantaneity, the hyperspectral remote sensing is a possible effective means to judge the leakage of CO₂ geological storage by the spectral change characteristics of vegetation.

The morphological characters and anatomic structure of plant leaf has an extremely active change response and adaptation to environmental factors. By revealing the hyperspectral curve response of soybean leaf stressed by CO₂ leakage condition, this paper found out the significant change of the spectral characteristics in visible, infrared and near-infrared bands, which may have something to do with adaptive changes in the leaf shape, epidermis characteristics and basic anatomical structure, etc. Some studies have demonstrated that, under the condition of CO₂ with high concentration, the leaf thickness often presents an increasing tendency, wherein the change trends may vary due to the different thickness of tested plant epidermis, mesophyll parenchyma and collenchymas as well as the proportion taken account in the total thickness [22]. The morphological characters and anatomic structure of plant leaf from different photosynthetic pathways have a difference on the response to CO₂ with high concentration. The multiplied CO₂ concentration would significantly increase the C₃ leaf thickness and reduce the number of top surface porosity and remain insignificant changes on C₄ leaf thickness and increase the number of surface porosity. Besides, the chlorophyll content of C₃ leaf as well as the number and volume of chlorophyllin in bundle sheath cells were reduced to a great extent than that of C₄ leaf [23]. Upon the analysis for hyperspectral data stressed by the CO₂ leakage, this study has demonstrated that the chlorophyll content of soybean leaf would present an obvious reduction trend as the stress level enhances, wherein such change trend has something to do with the CO₂ stress response. The existing studies on morphological anatomy of soybean leaf is not sufficient, so this part should be appropriately added in future work based on the spectral data to conduct the corresponding analysis with spectral characteristics changes.

CONCLUSIONS

This paper calculated and obtained spectral feature parameters related to the soybean stressed by CO₂ leakage by measuring the hyperspectral data and chlorophyll contents of soybean leaf stressed by CO₂ leakage. By the stepwise regression analysis of spectral feature parameters and chlorophyll content, this paper obtained the change law that can be used to analyse the chlorophyll content with hyperspectral

remote sensing and thus analysed the extent of CO₂ leakage stress.

The reflection spectrum feature analysis for soybean leaf stressed by CO₂ leakage has shown that, the absorption depth, normalized reflection at absorption valley, normalized reflection at green peak, red edge position, red shoulder position, absorption width and degree of symmetry of spectral characteristic parameters for soybean leaf during the mature period have a definite relation with the change of CO₂ leakage stress and have no definite numerical relationship with the absorption valley position and green peak position.

The multiple stepwise regression analysis for chlorophyll content by using the spectral feature parameters has shown that, chlorophyll content respectively has a negative and positive correlation with the normalized reflection at green peak and red edge wavelength, whose inversion equation is $Y = -19575 + 11.9X_1 - 45.119X_2 + 17.573X_3$.

It has indicated that, the inversion model for hyperspectral chlorophyll established based on the stepwise regression can be used to reflect the relation between the hyperspectral reflectance curve and chlorophyll content.

Mastering the chlorophyll content and spectrum characteristics of soybean leaf stressed by CO₂ leakage, especially the normalized reflection at green peak and red edge position, may be conducive to analyse the extent of stress on the soybean, offering theoretical foundations for CCS project site selection, monitoring and evaluation of ground surface eco-environment and remote sensing CO₂ leakage points, etc.

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