SPECTRAL RESPONSE OF WHEAT (*TRITIZNM AESTIVUM* L.) LEAVES TO COPPER STRESS

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1. INTRODUCTION

Cu is required by plants for normal growth and development, while it is also a heavy metal which has its inherent toxicity. Cu deposits in the soils by bedrock weathering processes and human activities such as mining and smelting cause serious contamination of terrestrial environment. This research focused on the relationship between spectral variation and Cu contents in leaves while the plant uptakes excess Cu.

2. MATERIALS AND METHODS

2.1. Water culture experiment

Wheat (*TritiZnm aestivum* L.) seeds were sterilized with a 5% NaClO solution, germinated in thermostat at (25±2)˚C after being washed with deionized water, and cultured in a culture solution 36h later. The water culture experiment was conducted in a plastic water trough. Each trough contained 20 transplanted seedlings and was placed on a culture shelf conditioned with a temperature of (25±1) ˚C, an illumination intensity of 200μmol $\cdot m^{-2} \cdot s^{-1}$ and a light-dark ratio of 12h/12h. 4 d later, the plants were cultured in Hoagland's nutrient solution and treated with $CuSO_4$ solution. 6 treatments were installed: 0, 2.5, 5, 10, 20 and 40 mg $\cdot L^{-1}$ of $CuSO_4$. During the experiment, the culture solution was aerated for 2 h per day, and replaced weekly. The spectra, chlorophyll contents and Cu contents of wheat leaves were measured after a 28 d successive Cu stress.

2.2. Data collection

Reflectance spectra was collected using an ASD Fieldspec FR Spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) with a wavelength range of 350-2500 nm, a spectral resolution of 3 nm between 350-1000 nm and 10 nm between 1000-2500 nm. The spectrometer is equipped with an Integrating Sphere (Li-Cor1800, Lincoln, NE, USA). Every spectral measurement was an average of 20 scans of 10 leaves sampled from every plant. The reflectance of wheat (*TritiZnm aestivum* L.) were calculated by equation 1, where f is the reflectance of wheat leaves, f_r is the reflectance of white reference, DN_r is the average DN of white reference, DN is the average DN of wheat leaves, DN_0 is the average DN of stray light of Integrating Sphere.

$$f = \frac{f_r(DN - DN_0)}{(DN_r - DN_0)} \tag{1}$$

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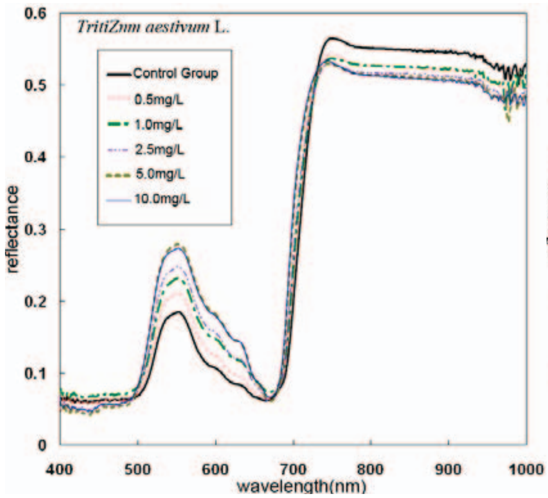


Fig. 1. Spectra of *TritiZnm aestivum* L. leaves under different contents of Cu stress

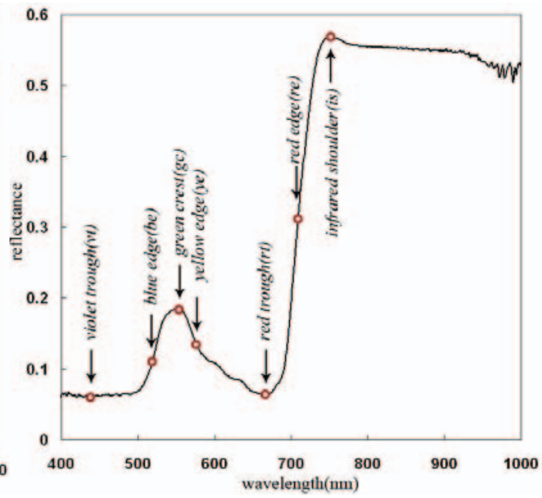


Fig. 2. Characteristic wavelengths of reflectance

The chlorophyll contents of wheat leaves were measured using a SPAD-502 chlorophyll meter, the mean chlorophyll index was calculated by the average of measured chlorophyll data of 10 leaves.

The *TritiZnm aestivum* L. roots and leaves were collected, cleaned and dried, then processed using the concentrated solution of HNO_3 and $HClO_4$. The copper concentration in the digestive solution was determined by atomic absorption spectrophotometer (AAS, Hitachi 180-80, Japan) under the condition: a wavelength of 324.8 nm, a slit width of 1.3 nm, an acetylene runoff of 2.0L $\cdot min^{-1}$ and an electric current of 7.5 mA.

2.3. Determination of characteristic wavelengths

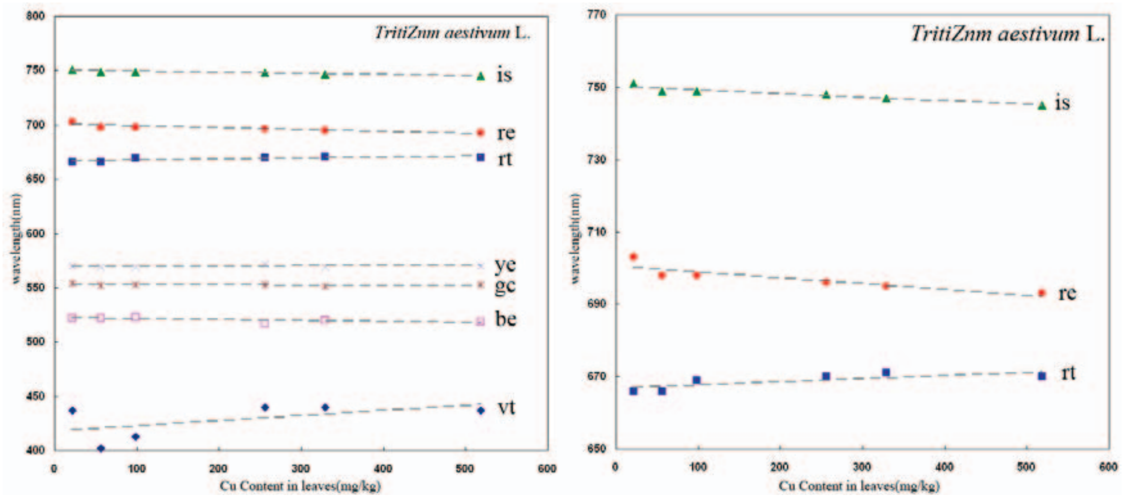
Seven characteristic wavelengths (Fig. 2) are selected to analyze the spectral variation of *TritiZnm aestivum* L. leaves: (1) violet trough (λ_{vt}: wavelength of the minimum value of reflectance from 382 nm to 500 nm), (2) blue edge (λ_{be}: wavelength of the maximum value of the first-order derivative of the reflectance from 450 nm to 550 nm), (3) green crest (λ_{gc}: wavelength of the maximum value of the reflectance from 500 to 600 nm), (4) yellow edge(λ_{ye}: wavelength of the minimum value of the first-order derivative of the spectrum from 550 to 650 nm), (5) red trough (λ_{rt}: wavelength of the minimum value of the spectrum from 600 to 720 nm), (6) red edge(λ_{re}: wavelength of the maximum value of the first-order derivative of the reflectance from 670 to 780 nm), (7) infrared shoulder (λ_{is}: wavelength of the maximum value of the reflectance from 750 to 950 nm).

2.4. Spectral dissimilar index and correlation coefficient

The spectral dissimilar index can be considered as an indicator of spectral variation (equation 2), where the $I_{dis,\lambda}$ is dissimilar index of wavelength λ, $r_{Cu,\lambda}$ is the reflectance under Cu stress of wavelength λ, $r_{con,\lambda}$ is the reflectance of control group of wavelength λ which can be seen as the reference level of the data.

$$I_{dis,\lambda} = \frac{r_{Cu,\lambda} - r_{con,\lambda}}{r_{con,\lambda}} \qquad (2)$$

The spectral correlation coefficient δ_λ between reflectance of wavelength λ and Cu contents of *TritiZnm aestivum* L. leaves is also calculated.



(a) 7 characteristic wavelengths (b) 3 characteristic wavelengths around red edge

Fig. 3. The relationship between the characteristic wavelengths and the Cu contents in leaves

2.5. FluorMODleaf model and fluorescence emission analysis

FluorMODleaf model is used to analyze to fluorescence effect of *TritiZnm aestivum* L. leaves under Cu stress. FluorMODleaf [1] is a new leaf fluorescence emission model based on the PROSPECT model. The input variables of FluorMODleaf are: the number of elementary plates N , also called leaf structure parameter, the total chlorophyll content C_{ab} , the total carotenoid content C_{cx} , the equivalent water thickness C_w , and the dry matter content C_m (or leaf mass per area), as in the new PROSPECT-5, plus the $\sigma_{\mathrm{I}}/\sigma_{\mathrm{II}}$ ratio referring to the fluorescence quantum efficiency of Photosystem I (PS I) and Photosystem II (PS II), τ_{I} and τ_{II} are mean fluorescence lifetimes of PS I and PS II . Inversion of the model can be performed by equation 3 [2]. Where $X = (x_1, x_2, ..., x_n)$ is the vector of model input parameters, m is the number of the measured reflectance values ρ_j^* , ρ_j is the model reflectance value on the boundary of the given region, ω_i is a weight, $\omega_i = 0$ in the given region $x_i \in [x_{i,\min}, x_{i,\max}]$ and ω_i = const else, $x_{e,i}$ is the expert estimate of the parameter x_i , and dx_i is a tolerance for the parameter x_i which controls the sensitivity of the merit function on the expert estimate. This procedure is optimized by Powell algorithm.

$$F(X) = \sum_{j=1}^{m} \left(\frac{\rho_j^* - \rho_j}{\varepsilon_j} \right)^2 + \sum_{i=1}^{n} \left[(x_i - x_{i,b})^4 \omega_i^2 + \left(\frac{x_i - x_{e,i}}{dx_i} \right)^2 \right] \tag{3}$$

Tab. 1. List of Cu contents in *TritiZnm aestivum* L.

No.	Added $CuSO_4$ ($mg \cdot L^{-1}$)	Cu contents in leaf and root ($mg \cdot kg^{-1}$)		Transfer factor (L/R)	Root length (cm)	Chlorophyll contents index (SPAD)
		C_L (leaf)	C_R (root)			
S1	0.0	22	28	—	22.1	39.5
S2	0.5	56	392	0.14	20.5	38.8
S3	1.0	98	605	0.16	18.1	38.5
S4	2.5	256	1568	0.16	16.5	38.3
S5	5.0	329	1732	0.19	12.1	36.2
S6	10.0	518	2285	0.23	16.5	38.3

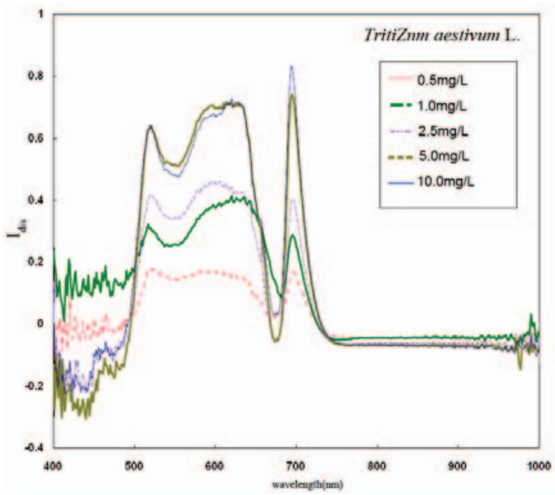


Fig. 4. The dissimilar index

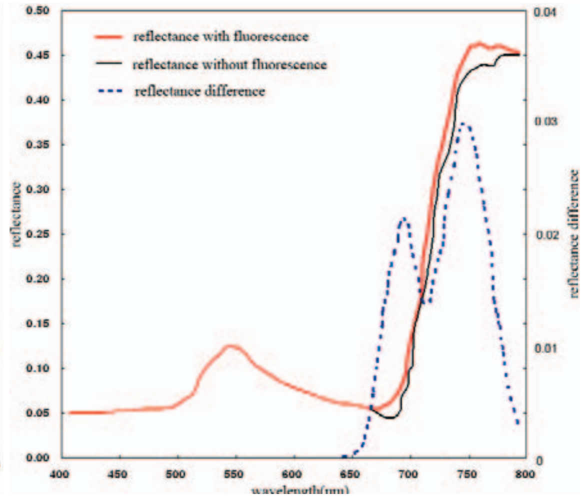


Fig. 5. The fluorescence emission effect (after P. J. Zarco-Tejada)

3. RESULTS AND DISCUSSION

The measured reflectance spectra of different groups are shown in Fig.1, and the chlorophyll and Cu content is shown in Tab. 1. The relationship between characteristic wavelengths and Cu contents in leaves is illustrated in Fig. 3. The blue shift of red edge is about 10 nm, the blue shift of red shoulder is about 5 nm, and the red shift of red trough is about 5 nm. The dissimilar index is shown in Fig. 4. The dissimilar index illustrates that there is a strong positive correlation between spectral variation and Cu contents of leaves in the visible region, and a negative correlation in the near-infrared region. The dramatic increase of reflectance at about 685 nm indicates that the PSⅡ is related to Cu stress, while it has been reported that the PSⅠ emission spectrum displays a single broad band at 740 nm, and the main peak of PSⅡ emission spectrum is at 683 nm. The inversion results of the FluorMODleaf model demonstrate that the reflectance is affected by fluorescence emission seriously (Fig. 5).

From the results above, it can be concluded that the spectral variation of *TritiZnm aestivum* L. leaves are caused by: (1) The excess of copper induced the inhibition of pigment synthesis and affected the development of the photosynthetic apparatus, which is one of the reasons why the reflectance increases in the visible region; (2) The decreased reflectance in the near-infrared, due to damage to leaf cell walls and mesophyll tissue. (3) The fluorescence effect is also an important factor that affects the reflectance spectra of leaves from 600 to 800 nm.

4. REFERENCES

[1] R. Pedrós, Y. Goulas, S. Jacquemoud, et al, “FluorMODleaf: A new leaf fluorescence emission model based on the PROSPECT model”, *Remote Sensing of Environment*, vol. 114, pp. 155-167, 2010.

[2] D. S. Kimes, Y. Knyazikhin, J. L. Privette, et al, “Inversion methods for physically-based models”, *Remote Sensing Reviews*, vol. 18, pp. 381-439, 2000.