Non-destructive measurement of chlorophyll in tomato leaves using spectral transmittance

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Abstract: In this study, spectral transmittances were measured in the wavelength range from 300 nm to 1100 nm of tomato leaves with different chlorophyll contents and compositions, and the correlations between the spectral transmittances and the contents of chlorophyll a, chlorophyll b and total chlorophyll were analyzed. With the characteristic wavelengths of 560 nm, 650 nm, 720 nm and the reference wavelength of 940 nm, nine sets of characteristic spectral parameters were obtained. According to the results of correlation analysis and regression model exploration, characteristic spectral parameters of T940/T560, T940/T650 and log (T940/T560) among the nine sets of parameters were highly correlated to the estimated contents of chlorophyll a, chlorophyll b and total chlorophyll of tomato leaves. The relative errors of total chlorophyll and chlorophyll a/b ratio were (5.1±3.7)% and (4.9±4.3)%, respectively. Therefore, the above three characteristic spectral parameters could be applied in the rapid non-destructive estimation of the contents of chlorophyll a, chlorophyll b and total chlorophyll as well as chlorophyll a/b ratio of tomato leaves.

Keywords: chlorophyll, characteristic wavelength, characteristic spectral parameter, spectral transmittance

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1 Introduction

Chlorophyll is an important pigment for photosynthesis of plants and its content and composition directly affect the photosynthetic capacity, nutrient level, growth and development of plants. The contents of chlorophyll a and chlorophyll b as well as their proportion directly affect the selective light absorption and utilization in plants[1-3]. Compared with total chlorophyll content, the contents of chlorophyll a and chlorophyll b could better reflect the photosynthetic capacity and growth status of plants. The changes in temperature, light, water, fertilizer and other environmental conditions directly affect the synthesis of chlorophyll[4-6]. Bednarz and Oosterhuis[7] found that the contents of chlorophyll a, chlorophyll b and total chlorophyll as well as chlorophyll a/b ratio decreased by 45%, 25%, 39% and 26%, respectively, in the leaves of the cotton plants, which were under the potassium deficiency status for 19 d, compared with those in the plants under normal potassium level. Zhang and Shang[8] studied the pepper seedlings under the conditions of weak lighting and salt stress for 1 d, 5 d, 9 d and 15 d, and found that the contents of total chlorophyll respectively increased by 2%, 12%, 22% and 35%, and chlorophyll a/b ratios decreased by 0.7%, 1.5%, 4.4% and 6.9%, respectively. Therefore, rapid quantitative

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measurement of the composition and content of chlorophyll in leaves is conducive to grasping the physiological status of plants, which can be used to guide plant production management.

The extraction-based colorimetric method can be used to accurately measure the chlorophyll content in plant leaves, but it is cumbersome and time-consuming and cannot meet the management requirements of modern agricultural production[9]. Spectrum signals are responsive to both biochemical contents and structural properties of plant leaves[10-12]. In 1960s, Swidler and Benedict[13] started the spectroscopic study on the non-destructive measurement of the chlorophyll content in leaves. In recent years, with the development in the spectrometry technology, the rapid non-destructive measurement method based on the transmission and reflectance spectra characteristics has been widely applied to measure the total chlorophyll content in the leaves of rice[14], wheat[15], maize[16], soybean[17], tomato[18], potato[19] and apple[20], but it is difficult to directly measure the contents of various chlorophyll components with the above described method. In this study, , based on the transmission and reflectance spectra characteristics of tomato leaves, the characteristic spectral parameters were selected for the quantitative estimation of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl), and chlorophyll a/b ratio (Chl a/b) to achieve rapid non-destructive quantification of chlorophyll composition.

2 Materials and methods

2.1 Plant materials and cultivation methods

Mature leaves of tomato plants (*Lycopersicon esculentum* Mill., cv. Fengshou) cultivated in matrix were selected as plant materials. Tomato seedlings were planted in 12 cultivation containers (130 cm × 90 cm × 25 cm) in an environment-controlled solar greenhouse in May 2013 and nine seedlings were planted in each container. The cultivation matrix was prepared with vermiculite, peat and perlite according to the ratio of 3:1:1. According to Japanese Yamazaki tomato nutrient solution formulation, the contents of main nutrition elements were unchanged except that the contents of nitrogen and potassium were adjusted. In the experiment, six plots were arranged (Table 1). The nutrient solution was regularly irrigated daily. After planting, the irrigation capacity was arranged as follows: 200 mL/plant in the first two weeks, 400 mL/plant from the 3rd to the 4th week, and 800 mL/plant from the 5th to the 12th week. The solar greenhouse was equipped with the pad and fan cooling ventilation device, internal and external shading devices, supplemental lighting device, and CO_2 regulation device for automatic environment control. From the 8th to the 12th week after planting, tomato leaves were sampled weekly for characteristic spectral scanning and the measurement of chlorophyll contents. The 4th or 5th leaf from the plant bottom was sampled.

Table 1 Concentrations of main nutrition elements in different nutrient treatments

Treatments	Main nutrition elements concentrations/mM					
	T0	T1	T2	T3	T4	T5
NO_3	0	0	9	9	18	18
NH_4	0	0	0.5	0.5	1	1
P	1	1	1	1	1	1
K	0	8	0	8	0	8
Ca	7.5	5.5	3.5	3.5	3.5	3.5
Mg	2	2	2	2	2	2

2.2 Characteristic spectral scanning and chlorophyll content measurement of tomato leaves with the extraction-based colorimetric method

The spectral transmittance of tomato leaves was determined with large sample compartment and integrating sphere of a spectrophotometer (UV3150, Shimadzu, Japan). The scanning wavelength range was from 300 nm to 1 100 nm and the scanning step was 1 nm. The grating switching point was 820 nm. For each leaf, spectral reflectance was measured for twice on both sides of the middle of the main vein and the means of four measurements were used as the result. In the experiment, 116 leaves were sampled and the chlorophyll contents were measured with the extraction-based colorimetric method immediately after spectral scanning.

In the extraction-based colorimetric measurement of chlorophyll content, tomato leaves were firstly cut into pieces. Then approximately 0.1 g cut leaf sample was weighed and added into a 15 mL plastic tube with a stopper. Then, 10 mL of 80% acetone was added into the tube for 48 h extraction in the dark. The supernatant was acquired to measure its absorbance at 663 nm and 645 nm with the spectrophotometer. According to Arnon's calibration equation, the contents of Chl a and Chl b were measured and then TChl content and Chl a/b were calculated.

2.3 Data processing and estimation methods

With SPSS software, the correlations between spectral transmittance (300-1100 nm) and chlorophyll contents of each tomato leaf sample were analyzed to determine the characteristic wavelength and reference wavelength. Based on the nine sets of characteristic wavelength and reference wavelength, the characteristic spectral parameters were obtained to detect chlorophyll contents and composition in tomato leaves for measurement verification.

3 Results and discussion

3.1 Contents and composition of chlorophyll in leaf samples

After collecting 116 leaves, where 95 leaves were randomly selected as the calibration set and 21 leaves were selected as the validation set. Then the correlation analysis was made between spectral transmittance and chlorophyll contents. In the calibration set, Chl a content ranged from 0.69 mg/g to 2.14 mg/g, while Chl b content ranged from 0.23 mg/g to 1.31 mg/g, and TChl content ranged from 1.09 mg/g to 3.42 mg/g. In the validation set, Chl a content ranged from 0.94 mg/g to 2.11 mg/g; Chl b content ranged from 0.43 mg/g to 1.31 mg/g; TChl content ranged from 1.33 mg/g to 3.24 mg/g. According to the results of Kolmogorov-Smirnov test, chlorophyll contents of the calibration set and validation set showed a normal distribution (Table 2).

Table 2 Statistic data of Chl a, Chl b and TChl contents in tomato leaves (mean ± standard deviation).

		Sample quantities	Mean values /mg·g^{-1}	Maximum values/mg·g^{-1}	Minimum values/mg·g^{-1}
Calibration Samples	Chl a	95	1.49±0.34	2.14	0.69
	Chl b	95	0.72±0.23	1.31	0.23
	TChl	95	2.17±0.59	3.42	1.09
Validation Samples	Chl a	21	1.57±0.35	2.11	0.94
	Chl b	21	0.79±0.25	1.31	0.43
	TChl	21	2.36±0.60	3.24	1.33

3.2 Correlation between transmission spectral characteristics and chlorophyll contents of tomato leaves

Transmission spectra of tomato leaves are formed under absorption, reflection, and transmission among the surface structure, internal structure and internal biochemical compositions[21,22]. Reflection spectra of tomato leaves showed the similar spectral characteristics to the leaves of green plants. In the visible spectral region of 400-700 nm, because the chlorophyll exhibits strong absorption to red light and blue light and the relatively weak absorption to green light, the low transmission zone, valley and peak were respectively formed in the ranges of 400-500 nm (blue light), 600-700 nm (red light) and around 550 nm (green light). Tomato leaves exhibited strong infrared reflection characteristics in the near-infrared region (800-1100 nm). The reflectance was about 50% and the absorptivity was less than 10%. Thus, the transmittance reached 40%-50%.

The 95 sample leaves were divided into five groups according to different average total chlorophyll contents (1.09 mg/g, 1.44 mg/g, 2.01 mg/g, 2.43 mg/g and 3.23 mg/g). The spectral transmittance distribution of each group of leaves is shown in Figure 1.

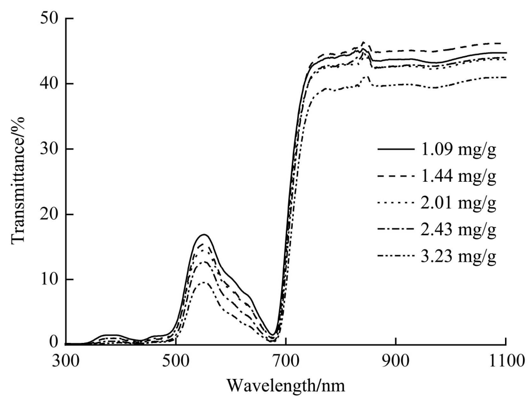

Figure 1 Leaf transmittance properties of tomato leaves with different chlorophyll contents

In the visible light range, the low transmission zone, valley and peak are respectively formed in the range of 400-500 nm, at 680 nm and around 550 nm. With the increase of the TChl content in leaves, the absorption of visible light increases. Therefore, the spectral transmittance within the band decreases. In the near-infrared band, the spectral transmittance of leaves is mainly affected by the surface characteristics, internal structure and other factors of leaves. The influence of chlorophyll content on the spectral transmittance is small. The changing trend of the transmittance spectra in the near-infrared band is different from that in the visible light region. These results are consistent with previous results of the characteristic spectra of plant leaves[23,24].

The contents of Chl a, Chl b and TChl and Chl a/b were significantly negatively correlated to spectral transmittance of tomato leaves, which showed the similar trends in different bands. In the visible spectral range, the correlation coefficient was between −0.8 and −0.7 in the region of 400-500 nm and between −0.9 and −0.8 in the region of 500-700 nm. However, the correlation coefficient in the vicinity of 680 nm was higher than −0.8. In the near-infrared region, with the increase in the wavelength, the correlation coefficient gradually decreased. In the region of 700-1100 nm, the correlation coefficient was between −0.7 and −0.4 (Figure 2).

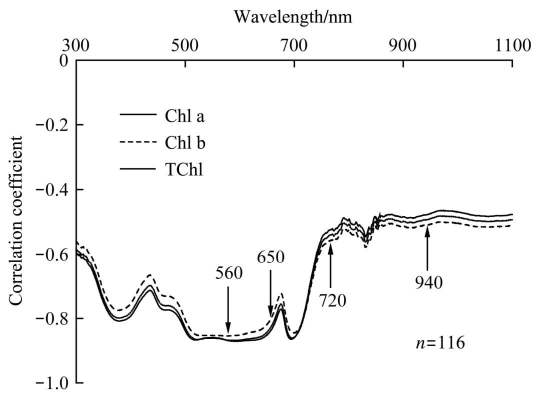

Figure 2 Correlation coefficients between spectral transmittance and the contents of Chl a, Chl b, and TChl

3.3 Determination of the characteristic wavelength and characteristic spectral parameters

In order to eliminate the influences of inhomogeneity of tomato leaf samples and random noise of a spectrometer on spectral data analysis and increase the estimation precision of chlorophyll content, the ratio and the difference between the spectral parameters of characteristic bands and the spectral parameters of the reference band were used as the characteristic parameters[25]. Ding et al.[26] selected the spectral absorbance at 440 nm, 500 nm and 680 nm in the blue band, green band, red band and near-infrared band, and the spectral reflectance at 550 nm and 700 nm as the characteristic spectral parameters to estimate the chlorophyll content of tomato leaves. Andrew et al.[27] found that the most suitable characteristic spectral parameters for the estimation of the chlorophyll contents in the leaves of birch were the spectral reflectance at 705 nm and 750 nm. In this paper, the correlation between spectral transmittance and the contents of Chl a, Chl b, and TChl was studied. The selected characteristic wavelength included 560 nm in the green band, 650 nm in the red band, 720 nm in red edge band as the characteristic wavelength, and the reference wavelength was 940 nm in the near-infrared band. Because no obvious characteristic peak or valley was observed in the blue region and spectral data this region had the low correlation with the chlorophyll content, no characteristic wavelength was selected from the blue region.

Nine sets of characteristic spectral parameters were selected for non-destructive quantitative estimation of the contents of Chl a, Chl b, and TChl. The correlation coefficients between the nine sets of characteristic spectral parameters and the content of Chl a was higher than 0.92. The correlation coefficients between Chl b content and various characteristic spectral parameters (T940/T560, T940/T650, log(T940/T560), log(T940/T650), and (T940−T560)/(T940+T560) were higher than 0.89. The correlation coefficients between the nine sets of characteristic spectral parameters and the TChl content were higher than 0.90 (Table 3). Therefore, five characteristic spectral parameters (T940/T560, T940/T650, log(T940/T560), log(T940/T650), (T940−T560)/(T940+T560)) with higher correlation coefficients were selected to establish the estimation model.

Table 3 Correlation coefficients between characteristic spectral parameters and the contents of Chl a, Chl b, and TChl

Characteristic parameters	Correlation coefficients (R^2)		
	Chl a content	Chl b content	TChl content
T940/T560	0.95	0.92	0.95
T940/T650	0.94	0.91	0.93
T940/T720	0.92	0.89	0.92
log(T940/T560)	0.95	0.92	0.94
log(T940/T650)	0.94	0.89	0.93
log(T940/T720)	0.92	0.89	0.92
(T940−T560)/(T940+T560)	0.95	0.92	0.94
(T940−T650)/(T940+T650)	0.92	0.87	0.91
(T940−T720)/(T940+T650)	0.92	0.89	0.92

3.4 Estimation precision of chlorophyll contents

Determination coefficients (R^2) of estimation regression models for the contents of Chl a, Chl b, and TChl established with the above five sets of characteristic parameters were greater than 0.84. The characteristic parameter, log(T940/T560), allowed the more precise estimation regression models for the contents of Chl a and TChl (R^2 were respectively 0.91 and 0.90). Estimation regression model for the content of Chl b established with T940/T560 had the higher precision (R^2 was 0.88). The linear regression relationships between five sets of characteristic parameters and model variables were significant (Table 4).

Table 4 Coefficients of determination (R^2) for regression models of Chl a, Chl b, and TChl contents

Characteristic parameters	Coefficients of determination (R^2)		
	Chl a	Chl b	TChl
T940/T560	0.91	0.89	0.90
T940/T650	0.88	0.87	0.89
log(T940/T560)	0.91	0.88	0.90
log(T940/T650)	0.88	0.84	0.88
(T940−T560)/(T940+T560)	0.90	0.87	0.90

With the validation sample set of 21 tomato leaves, the contents of Chl a and TChl with characteristic spectral parameters have been estimated and the estimation results with the measured results to verify the reliability of the estimation model have been compared. Then, Chl a/b and the calculated results were compared with the measured results to verify the precision of the estimation model of Chl a/b.

With the five sets of characteristic spectral parameters, T940/T560, T940/T650 and log (T940/T560), the precise estimation was obtained. The relative errors of the estimated content of Chl a, Chl b, and TChl were less than 6.8% (Table 5). The relative error of the estimated Chl a/b was less than 4.9%. These results showed that the characteristic spectral parameters (T940/T560, T940/T650 and log(T940/T560)) allowed the precise estimation of the contents of Chl a, Chl b, and TChl as well as Chl a/b in tomato leaves with the relative low errors, which could meet the requirements of rapid non-destructive measurement of the chlorophyll content and composition.

Table 5 Relative errors of Chl a, Chl b, TChl content, and Chl a/b ratio estimated based on characteristic spectral parameters (mean ± standard deviation)

Characteristic parameters	Relative errors/%			
	Chl a	Chl b	TChl	Chl a/b
T940/T560	4.4±3.4	6.4±5.6	5.0±3.8	4.3±4.1
T940/T650	5.2±4.1	6.7±5.9	5.1±3.7	4.9±4.3
log(940/560)	4.6±4.2	6.8±4.9	4.9±4.2	4.7±4.3
log(T940/T650)	4.5±4.6	7.4±6.9	4.8±3.9	4.9±4.6
(T940−T560)/(T940+T560)	5.3±5.1	7.3±6.5	5.9±4.1	5.0±4.4

4 Conclusions

Based on the correlation between the spectral transmittance in the band of 300-1100 nm and the contents of Chl a, Chl b and TChl in the leaves of tomato cultivated in greenhouse, characteristic wavelengths included 560 nm, 650 nm and 720 nm and the reference wavelength of 940 nm were selected, and T940/T560, T940/T650 and log(T940/T560) as the characteristic spectral parameters were established for estimating the contents of Chla, Chl b, TChl. The estimation of the contents of Chl a, Chl b, and TChl had the relative errors less than 6.8% and the estimation of Chl a/b had the estimation error less than 4.9%. The estimation model provided a new method and basis for the rapid non-destructive measurement of the contents of Chl a, Chl b, and TChl as well as Chl a/b. Therefore, this model established with characteristic spectral parameters can provide a theoretical basis for developing a portable chlorophyll detector. However, whether the established estimation model is applicable to the determination of chlorophyll contents of other plants should be verified, because the present study is only examined in tomato leaves.

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[References]

[1] Pandey D M, Kim K H, Yeo U D. Dynamic changes of photosynthetic pigments in soybean callus under high irradiance. Photosynthetica, 2003; 41(2): 311−314.

[2] Pilarski J, Kocurek M. The content of photosynthetic pigments and the light conditions in the fruits and leaves of sweet pepper. Acta Physiologiae Plantarum, 2005; 27(2): 173−182.

[3] Tokarz K, Pilarski J. Optical properties and the content of photosynthetic pigments in the stems and leaves of the apple-tree. Acta Physiologiae Plantarum, 2005; 27(2): 183−191.

[4] Anderson J M, Chow W S, Goodchild D J. Thylakoid membrane organization in sun shade acclimation. Australian Journal of Plant Physiology, 1988; 15(1-2): 11−26.

[5] Schoefs B, Bertrand M, Lemoine Y. Changes in the photosynthetic pigments in bean leaves during the first photoperiod of greening and the subsequent dark-phase. Comparison between old (10-d-old) leaves and young (2-d-old) leaves. Photosynthesis Research, 1998; 57(2): 203−213.

[6] Chu Z X, Tong Z, Feng L J, Zhang Q, Wen X G, Song S T, et al. Effect of different light quality on photosynthetic characteristics of cucumber leaves. Acta Botanica Sinica, 1999; 41(8): 867−870.

[7] Bednarz C W, Oosterhuis D M. Physiological changes associated with potassium deficiency in cotton. Journal of Plant Nutrition, 1999; 22(2): 303−313.

[8] Zhang Z G, Shang Q M. Photosynthetic characteristics of pepper leaves under low temperature, weak light and salt stress. Scientia Agricultura Sinica, 2010; 43(1): 123−131. (in Chinese with English abstract)

[9] Jiao W J, Min Q W, Lin K, Zhu Q K, Zhang J J. Progress and perspective on nutrition diagnosis of plant nitrogen. Chinese Agricultural Science Bulletin, 2006; 22(12): 351−355. (in Chinese with English abstract)

[10] Roberts D A, Ustin S L, Ogunjemiyo S, Hinckley T M. Spectral and structural measures of northwest forest vegetation at leaf to landscape scale. Ecosystems, 2004; 7(5): 545−562.

[11] Houborg R, Boegh E. Mapping leaf chlorophyll and leaf area index using inverse and forward canopy reflectance modeling and SPOT reflectance data. Remote Sensing of Environment, 2008; 112(1): 186–202.

[12] Ustin S L, Gitelson A A, Jacquemoud S, Schaepman M, Asner G P, Gamon J A, et al. Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. Remote Sensing of Environment, 2009; 113(9): 67–77.

[13] Benedict H M, Swidler R. Nondestructive method for estimating chlorophyll content of leaves. Science, 1961; 133(346): 2015.

[14] Yue Y M, Wang K L, Li R, Liu B, Chen H S. New spectral indices for quantifying chlorophyll content of paddy rice. Journal of Food Agriculture & Environment, 2012; 10(3-4): 1279−1284.

[15] Babar M A, Reynolds M P, Van Ginkel M, Klatt A R, Raun W R, Stone M L. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Science, 2006; 46(3): 1046−1057.

[16] Daughtry C S T, Walthall C L, Kim M S, de Colstoun E B, McMurtrey J E. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sensing of Environment, 2000; 74(2): 229−239.

[17] Salas E A L, Henebry G M. Separability of maize and soybean in the spectral regions of chlorophyll and carotenoids using the Moment Distance Index. Israel Journal of Plant Sciences, 2012; 60(1-2): 65−76.

[18] Main R, Cho M A, Mathieu R, O'Kennedy M M, Ramoelo A, Koch S. An investigation into robust spectral indices for leaf chlorophyll estimation. Isprs Journal of Photogrammetry and Remote Sensing, 2011; 66(6): 751−761.

[19] Botha E J, Leblon B, Zebarth B, Watmough J. Non-destructive estimation of potato leaf chlorophyll from canopy hyperspectral reflectance using the inverted PROSAIL model. International Journal of Applied Earth Observation and Geoinformation, 2007; 9(4): 360−374.

[20] Merzlyak M N, Solovchenko A E, Gitelson A A. Reflectance characteristic spectral and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biology and Technology, 2003; 27(2): 197−211.

[21] Penuelas J, Filella I. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends in Plant Science, 1998; 3(4): 151−156.

[22] Thomas J R, Oerther G F. Estimating nitrogen content of sweet pepper leaves by reflectance measurements. Agronomy Journal, 1972; 64(1): 11−13.

[23] Bauerle W L, Weston D J, Bowden J D, Dudley J B, Toler J E. Leaf absorptance of photosynthetically active radiation in relation to chlorophyll meter estimates among woody plant species. Scientia Horticulturae, 2004; 101(1-2): 169−178.

[24] Thomas J R, Gausman H W. Leaf reflectance vs. leaf chlorophyll and carotenoid concentrations for eight crops. Agronomy Journal, 1977; (5): 799−802.

[25] He D X, Hu J X. Plant nutrition indices using leaf spectral transmittance for nitrogen detection. Transactions of the CSAE, 2011; 27(4): 214−218. (in Chinese with English abstract)

[26] Ding Y J, Li M Z, An D K, Li S Q. Prediction of chlorophyll content using spectral response characteristics of greenhouse tomato. Transactions of the CSAE, 2011; 27(5): 244−247. (in Chinese with English abstract)

[27] Richardson A D, Duigan S P, Berlyn G P. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist, 2002; 153(1): 185−194.