●Report

A correlation analysis on chlorophyll content and SPAD value in tomato leaves

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Abstract

To investigate relationship between tomato（*Solanum lycopersicum*）leaf chlorophyll content and Minolta SPAD-502 plus chlorophyll meter, we studied leaves at plant vegetative growth stage and reproductive growth stage, and conducted correlation analysis to establish most optimal function model. The results showed that the correlation of SPAD value and the content of chlorophyll a, chlorophyll b and total chlorophyll content were significantly correlated in tomato leaves. At plant vegetative growth stage, the optimal mathematic function for SPAD value and chlorophyll a, chlorophyll b and total chlorophyll were $y = 0.0006x^{1.924}$（$r = 0.785$）, $y = 0.0006x^{1.8009}$（$r = 0.756$）and $y = 0.2317e^{0.0406x}$（$r = 0.869$）respectively. At reproductive growth stage, the optimal function models were $y = 0.0236x - 0.0705$（$r = 0.856$）for chlorophyll a, $y = 0.2975e^{0.0127x}$（$r = 0.793$）for chlorophyll b and $y = 0.0306x + 0.1443$（$r = 0.869$）for total chlorophyll. All these data proved SPAD-502 can be an effective tool used for rapid and nondestructive estimation of leaf chlorophyll content in tomato.

Key words：Chlorophyll, Tomato, SPAD-502 plus, Correlation analysis

Introduction

Chlorophyll, a materials base for photosynthesis, is most important photosynthetic pigment. The content of chlorophyll is one of main index reflecting leaf photosynthesis ability and plant health condition（Monje and Bugbee, 1992; Peng et al., 1993; Porra et al., 1989; Singha and Townsend, 1989）. Traditional way to measure chlorophyll content usually need to extract leaf tissue with organic solvents such as acetone, ethanol, N, N-dimethyl formamide（Bruinsma, 1961; McKinney, 1941; Moran, 1982; Moran and Porath, 1980）. Although this laboratory analysis method is relatively accurate, however, extraction is laborious, destructive, time-consuming, and expensive（Monje and Bugbee, 1992; Singha and Townsend, 1989）. In the meantime, significant pigment losses may occur during the extraction and dilution and lead to a high variability in the results（Shoaf and Lium, 1976）.

SPAD-502 chlorophyll meter（Konica Minolta, Tokyo, Japan）is a simple, portable diagnostic tool that measures the greenness or the relative chlorophyll content of leaves（Marquard and Tipton, 1987）. By measuring the leaf transmittance in two wave bands（400−500 nm and 600−700 nm）, this device quantifies the relative amount of chlorophyll with a reading in arbitrary unit（SPAD-502 Chlorophyll Index）that is proportional to the leaf chlorophyll concentration（Madeira et al. 2003; Minolta Camera Co. Ltd., 1989; Sim et al., 2015）, which provides a substantial saving in time, space and resources. Due to this rapid, non-destructive method, SPAD-502 has been extensively used in agriculture. High correlations between SPAD-502 value and chlorophyll content have been shown for several species of maize（Zotarelli et al., 2003）, rice（Turner and Jund, 1991）, wheat（Reeves et al., 1993）, coffee（Netto et al., 2005）, muskmelon（Azia and Stewart, 2001）, and soybean（Monje and Bugbee, 1992）. On the other hand, some research evidence also presented mathematical relationships between SPAD-502 readings and leaf chlorophyll may vary with plant growth stage（Chapman and Barreto, 1997）, growing conditions（Bullock and Anderson, 1998; Campbell et al., 1990; Nascimento and Marenco, 2010）and genotype（Sibley et al., 1996）which brought out inherent limitations of chlorophyll meters.

In spite of the many studies related to chlorophyll content varies with growth conditions of tomato leaf（Al-aghabary et al, 2005; Blunden et al, 1996; Wu and Kubota, 2008; Takayama et al, 2006）, the research on relationship between tomato chlorophyll content and the SPAD-502 value in regarding of different growth stages is still in sufficient in literature. Mathematical correlation calculate between SPAD value and chlorophyll content can be important to optimize the advanced interpretations of data from the chlorophyll meter.

This study was carried out to determine if there was a correlation between tomato leaf chlorophyll content and SPAD value; build mathematical function to describe relationship between chlorophyll content in leaves of different plant stages and SPAD values and optimize model to provide a more precise, reliable and easier method

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reference for estimation of tomato leaf chlorophyll content.

Materials and Methods

Plant Material and Growth Conditions

Tomato Momotaro (*Solanum lycopersicum*) was generally cultivated in a greenhouse in the experimental field of the Graduate School of Horticulture, Chiba University, Kashiwa, Japan (35°53′37″ N, 139°56′53″ E) from November 2014 to March 2015. The greenhouse was a Venlo-type with double spans, oriented north and south, covered with an ethylene-tetra fluoroethylene film and equipped with air-conditioner for winter heating and supplemented with natural ventilation from the roof and side windows, which were operated automatically based on the air temperature inside the greenhouse. During the experiment, the daytime mean air temperature was 24–30° C, the night-time mean air temperature was 17–22° C, and the daily mean relative humidity was maintained above 60%. Although the CO_2 concentration in the canopy was not measured, it was assumed to be close to the outside level, based on measurements in the same season in another year (data not shown).

Seeds were germinated in trays containing sand, peat, and perlite at equal parts of each on November 1st, 2014. After 24 days and with three true leaves, the seedlings were transplanted to cultivation benches (9 m × 0.4 m × 0.7 m, length × width × height) at a density of 10 plants·m^{-2} and irrigated with nutrient solution (Yamazaki, 1978) with nutrient film technique (NFT). The pH of the nutrient solution and the electrical conductivity were monitored periodically and maintained at approximately 6.5 and 2.0–3.0 dS·m^{-1}, respectively.

Plants growth stage was distinguished into vegetative stage (from transplanting to anthesis came out, 28 days) and reproductive stage (from anthesis to fruit harvesting, 63 days). The second terminal leaflets of leaves on the fifth youngest node (Matsuda et al., 2014) were chosen for samples in two stages separately, each stages consisted of 3 repeats and each repeat consisted 3 leaves per plant and 10 plants.

SPAD-502 value measurement

Before measurement SPAD-502 meter was calibrated using the reading checker supplied by the manufacturer. Each leaf SPAD value obtained was the average of 10 readings (5 on each side of leaf midrib), and then for each extract, two chlorophyll determinations were performed (León et al, 2007).

Chlorophyll spectrophotometric measurement

Each leaf measured by SPAD-502 was carefully picked, labeled, and frozen in storage bags with ice. After all samples collection carried back to laboratory and crushed in grinder. Extractions were done using pre-refrigerated acetone. An aliquot of this extract was used to spectrophotometrically determine total chlorophyll and chlorophylls a and b contents (Bruinsma, 1963). Absorbance readings were carried out at 649 and 665 nm, and the results are expressed as mg of chlorophyll/ g fresh tissue (Ling et al, 2011). Equation used for calculation as below: Chl a = $13.95 \times N_{665} - 6.88 \times N_{649}$; Chl b = $24.94 \times N_{649} - 7.32 \times N_{665}$; T Chl = Chl a + Chl b (Chl a short for content of chlorophyll a; Chl b for chlorophyll b; T Chl for total chlorophyll content; N_{649}, N_{665} the reading of absorbance at 649 and 665 nm respectively).

Statistical analysis

Regression analysis and correlation analysis was done with Excel 2013 (Microsoft, USA) and SPSS 21 (IBM, USA).

Results

SPAD value and chlorophyll content

Fig. 1 and Fig. 2 showed the relationships between the SPAD-502 value and chlorophyll content of leaves at plant vegetative stage and reproductive stage. With increase of SPAD values, the chlorophyll content showed a trend of synchronous increase. In spite of plant stages, a liner mathematical model was fitted best in relationships between total chlorophyll content and SPAD value while it fitted worst in chlorophyll b and SPAD value, and correlation of chlorophyll a and SPAD value was a continuum between of the two above. This showed that in tomato leaf, content of chlorophyll a was much higher than chlorophyll b and it also correlated better with SPAD value than chlorophyll b.

Regression analysis of correlation of SPAD value and chlorophyll content

Table 1 showed at plant vegetative growth stage, different mathematic modelling functions, correlations between SPAD value, as x, chlorophyll content, as y, were significantly different and correlations changed with different categories. For content of chlorophyll a and chlorophyll b, the highest correlation occurred both in power model function, with $y = 0.0006x^{1.924}$ ($r = 0.785$) and $y = 0.0006x^{1.8009}$ ($r = 0.756$) respectively. However, index model function fitted best to total chlorophyll content as $y = 0.2317e^{0.0406x}$ ($r = 0.869$).

Table 2 showed at plant reproductive growth stage, different mathematic modelling functions, correlations between SPAD value, as x, chlorophyll content, as y, were also significantly different. Correlations between SPAD value and chlorophyll b were generally lower than two other categories. Different with vegetative stage leaf, highest correlation for chlorophyll a and total chlorophyll occurred

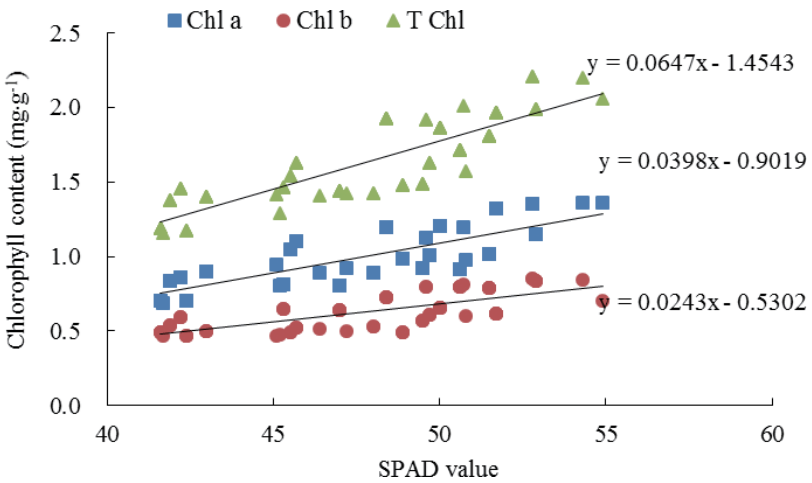

Fig. 1 Correlation of SPAD value and chlorophyll content in leaves at vegetative growth stage

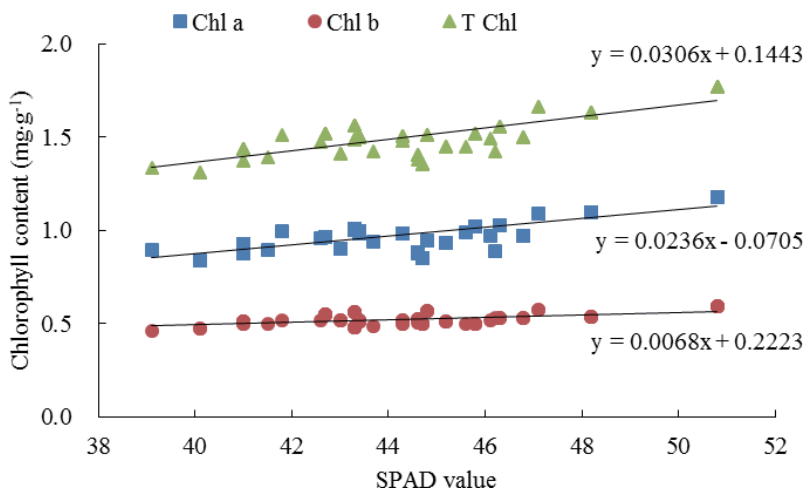

Fig. 2 Correlation of SPAD value and chlorophyll content in leaves at reproductive growth stage

Table 1 Regression analysis of correlations SPAD value with several mathematic models of chlorophyll content at vegetative growth stage

Category	liner model, $y = ax+b$	logarithmic model, $y = a\ln(x)+b$	Power model, $y = ax^b$	Index model, $y = ae^{bx}$
Chl a	$y = 0.0398x - 0.9019$ $R^2 = 0.5845$ r = 0.766 **	$y = 1.8668\ln(x) - 6.2107$ $R^2 = 0.5817$ r = 0.763**	$y = 0.0006x^{1.924}$ $R^2 = 0.6169$ r = 0.785 **	$y = 0.1388e^{0.041x}$ $R^2 = 0.6068$ r = 0.779 **
Chl b	$y = 0.0243x - 0.5302$ $R^2 = 0.5588$ r = 0.748**	$y = 1.134\ln(x) - 3.7496$ $R^2 = 0.5509$ r = 0.742 **	$y = 0.0006x^{1.8009}$ $R^2 = 0.5715$ r = 0.756**	$y = 0.0983e^{0.0386x}$ $R^2 = 0.5706$ r = 0.755 **
T Chl	$y = 0.0647x - 1.4543$ $R^2 = 0.7407$ r = 0.861**	$y = 3.0238\ln(x) - 10.048$ $R^2 = 0.7343$ r = 0.857**	$y = 0.001x^{1.9023}$ $R^2 = 0.7516$ r = 0.867**	$y = 0.2317e^{0.0406x}$ $R^2 = 0.7546$ r = 0.869 **

** means significant difference $p < 0.05$.

Table 2 Regression analysis of correlations SPAD value with several mathematic models of chlorophyll content at reproductive growth stage

Category	liner model, $y = ax+b$	logarithmic model, $y = a \ln(x) + b$	Power model, $y = ax^b$	Index model, $y = ae^{bx}$
Chl a	$y = 0.0236x - 0.0705$ $R^2 = 0.7327$ $r = 0.856^{**}$	$y = 1.0549\ln(x) - 3.0216$ $R^2 = 0.7174$ $r = 0.847^{**}$	$y = 0.0195x^{1.0321}$ $R^2 = 0.6889$ $r = 0.830^{**}$	$y = 0.35e^{0.0231x}$ $R^2 = 0.7005$ $r = 0.837^{**}$
Chl b	$y = 0.0068x + 0.2223$ $R^2 = 0.6006$ $r = 0.775^{**}$	$y = 0.3037\ln(x) - 0.6275$ $R^2 = 0.6037$ $r = 0.777^{**}$	$y = 0.0605x^{0.569}$ $R^2 = 0.5836$ $r = 0.764^{**}$	$y = 0.2975e^{0.0127x}$ $R^2 = 0.6288$ $r = 0.793^{**}$
T Chl	$y = 0.0306x + 0.1443$ $R^2 = 0.7551$ $r = 0.869^{**}$	$y = 1.3656\ln(x) - 3.6764$ $R^2 = 0.7396$ $r = 0.860^{**}$	$y = 0.0539x^{0.8771}$ $R^2 = 0.7225$ $r = 0.850^{**}$	$y = 0.6275e^{0.0196x}$ $R^2 = 0.7361$ $r = 0.858^{**}$

** means significant difference $p < 0.05$.

together in liner model function, with $y = 0.0236x - 0.0705$ ($r = 0.856$) and $y = 0.0306x + 0.1443$ ($r = 0.869$) respectively. For chlorophyll b, index model fitted best with function of $y = 0.2975e^{0.0127x}$ ($r = 0.793$).

Discussions

The regression coefficient between SPAD value and three chlorophyll parameters was highest in total chlorophyll content, both in vegetative stage and reproductive stage (Table 1 and 2). Since the wavelength for the leaf transmittance measurement in SPAD-520 was 600–700 nm, in which most chlorophyll absorb. Given that the variation in irradiation condition would cause changes in chlorophyll component, e.g. the ratio of chlorophyll a and chlorophyll b, the total chlorophyll content would be hardly affected by the fluctuation of chlorophyll a and chlorophyll b content, which contributed to a relatively stable coefficient to SPAD value. On the other hand, both the correlation analysis (showed in Fig. 1 and 2) and regression analysis (showed in Table 1 and 2) showed the coefficients were largely declined between SPAD value and chlorophyll b, compared to other two chlorophyll indexes. In leaf at vegetative growth stage, correlation of r in the optimal function was 3.6% lower than r of chlorophyll a and 13.0% lower than r of total chlorophyll. This trend also continue in leaf at reproductive growth stage, which reached 7.3% and 8.7% respectively. Given the fact that leaf condition would affect the accuracy of correlation analysis (Bullock and Anderson, 1998; Campbell et al., 1990; Chapman and Barreto, 1997), another important factor was related to SPAD-502 manufacture technique. The light wavelength designed for SPAD-502 chlorophyll meter was approximately 660 nm and peak absorbing wavelength of chlorophyll a was 662 nm while chlorophyll b was 644 nm, which indicated the excursion from 660 nm in chlorophyll b was 7 times higher than data in chlorophyll a. Due to less absorbing quantity, it explained why chlorophyll b correlated worse than chlorophyll a and total chlorophyll which was sum of these two indexes.

Regression analysis (showed in Table 1 and 2) presented different optimal mathematic function model of correlations between SPAD value and chlorophyll content based on coefficient value of r. Previous research usually utilized a single mathematic regression, liner model mostly, to regression analyze relationships between SPAD value and chlorophyll content (Hawkins et al, 2009; León et al, 2007; Marquard and Tipton, 1987; Netto et al, 2005; Schaper and Chacko, 1991; Yadava, 1986). These results are different to ours, which suggests that tomato has a different behavior from other species regarding the mathematical fit of the studied relationships. It was necessary to adjust research means according to specific plant and growth stage. Meanwhile the significant difference of coefficient values in single index analysis also suggested the importance and necessity of calculation method in estimation.

Conclusions

There was a significant relationship between SPAD-520 value and chlorophyll content in tomato leaves. But the optimized mathematical model for estimation chlorophyll content with SPAD value of leaves at different growth stages were different. With the highest correlation efficiency of four mathematic modelling functions at different growth stage, the results demonstrated that optimal model for chlorophyll a content was power function at vegetative stage and liner function at reproductive stage, which was opposite to total chlorophyll content, and to chlorophyll b content was power function at vegetative stage and index function at reproductive stage.

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