Relationship between chlorophyll content and soil plant analytical development values in two cultivars of fig (Ficus carica L.) as brassinolide effect at an open field

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Abstract. Relationship between chlorophyll and relative chlorophyll values obtained using soil plant analytical development (SPAD)-502 meter after brassinolide treatment were determined in two varieties of fig (Ficus carica L.) at an open field conditions using fresh weight basis. The experiment was arranged as split plot randomized complete block design (SRCBD) with four replications, and each experimental unit containing three plants. A different fig sample was considered as the main plot and brassinolide concentrations as sub-plots. There were four plants as destructive samples observed monthly for each replication. The correlation of SPAD value and the content of chlorophyll a, chlorophyll b and total chlorophyll content were significantly correlated in fig leaves. At IBT variety, the optimal mathematic function for SPAD value and chlorophyll a, chlorophyll b and total chlorophyll were Y=30.209e^{-0.081x} (r=0.6623), Y=388.42x-1.676 (r=0.3145), and Y=29.738e^{-0.072x} (r=0.5380) respectively. At MD variety, the optimal function models were Y=7.4524e^{-0.032x} (r=0.5318) for chlorophyll a, y=-0.0025x^2+0.1268x+0.2896 (r=0.3407) for chlorophyll b and Y=-0.0052x^2+0.1961x+3.1882 (r=0.5129) 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 fig.

1. Introduction
Chlorophyll is a main material for photosynthesis, found in plants, algae, and bacteria that gives them their green color and it also enables them to absorb the light for photosynthesis. The contents of chlorophyll reflect leaf photosynthesis ability and plant health condition [1, 2, 3, 4]. Traditional way to measure chlorophyll content usually need to extract leaf tissue with organic solvents such as acetone, ethanol, N, N-dimethyl formamide [5, 6, 7, 8]. Although this laboratory analysis method is relatively accurate, however, extraction is laborious, destructive, time-consuming, and expensive [1, 4]. In the meantime, significant pigment losses may occur during the extraction and dilution and resulted a high variability [9].

SPAD-502 chlorophyll meter (Minolta Inc, USA) is easy to use, non-destructive, and hand-held spectral device, portable diagnostic tool that measures the greenness or the relative chlorophyll content of leaves [10, 11, 12, 13, 14]. 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 [15, 16], which provides a substantial saving in time, space, resources and suitable for small plot areas.
Commonly, the SPAD meter clips on intact leaves and rapidly generates readings that lay from 0 to 50. Those readings are solely the indication of greenness, not the chlorophyll concentration [17]. Therefore, it is necessary to find a mathematical correlation between SPAD meter readings and the foliar chlorophyll concentration to foresee the chlorophyll amount in plant leaves [12]. Correlation ($R^2$) between SPAD-502 meters readings and extractable total chlorophyll (fresh weight basis) in tomato leaves was reported to be 0.87 [18]. The regression between total chlorophyll contents and SPAD readings of wheat, rice, and soybean leave samples extracted with dimethyl sulfoxide results a relationship ($R^2$) of 0.93 [1]. Likewise, a correlation ($R^2$) of 0.87 was found between fresh leaf tissue chlorophyll determined using solvent extraction methods and SPAD meter readings [14]. They also reported that chlorophyll values were differed by $\approx 6\%$ when SPAD values converted from radiometric to solvent extracted chlorophyll units.

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 rice [19], cotton [20], wheat [21], cherries [22], and sweet pepper [23]. On the other hand, some research evidence also presented mathematical relationships between SPAD-502 readings and leaf chlorophyll may vary with plant growth stage [24], growing conditions [25, 26, 27] and genotype [28] which brought out inherent limitations of chlorophyll meters.

Two fig varieties, Improved Brow Turkey (IBT) and Masui Dauphine (MD), have different effect on physiological changes especially in chlorophyll content after receiving different concentrations of brassinolide [29]. Brassinolide is one of the brassinosteroids, which are steroidal plant hormones showing a wide occurrence in the plant kingdom, that have unique biological effects on growth and development [30, 31].

In spite of the many studies related to chlorophyll content varies with growth conditions [32, 33, 34, 35], the research on relationship between fig chlorophyll content and the SPAD-502 value in leaf of fig is still insufficient 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 fig leaf chlorophyll content and SPAD value; build mathematical function to describe relationship between chlorophyll content in leaves and SPAD values and optimize model to provide a more precise, reliable and easier method reference for estimation of fig leaf chlorophyll content after receiving brassinolide.

2. Materials and methods

2.1. Plant material and growth conditions
Fig-planting materials were propagated using cutting methods taken from mature two- to three-year-old figs and transferred into media containing 3:2:1 mixed soil (top soil:organic matters:sand). Two different fig (IBT and MD) varieties were subjected to four levels (0, 50, 100 and 200 mL L$^{-1}$) of BL concentration. One-month-old fig tree seedlings were sprayed monthly with a solution of brassinolide according to the treatments. A different fig sample was considered as the main plot and BL concentrations as sub-plots. The experiment was arranged as a Split Plot Randomized Complete Block Design (SRCBD) with four replications. There were four plants as destructive samples observed monthly for each replication. The experiment was conducted in an open field at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia situated at 2° 58” N and 101° 44’ 04” E in Serdang, Selangor, Malaysia from May to December 2017. Data were recorded monthly.

2.2. SPAD-502 value measurement
Before measurement SPAD-502 meter was calibrated using the reading checker supplied by the manufacturer. The leaves of F. carica L. with different greenness (pale yellow, light green and dark green) were selected for analysis and total leaf chlorophyll content was analyzed. Each leaf SPAD value obtained was the average of 5 readings (3 on each side of leaf), and then sampled for chlorophyll determinations [36].
2.3. Chlorophyll spectrophotometric measurement

Each leaf measured by SPAD-502 was carefully picked and labeled. After that, all samples collection carried back to laboratory. Leaf discs 3 mm in diameter were obtained from the leaf sample using a hole puncher. The leaf disks were immediately immersed in 20 ml of 80% acetone in an aluminium foil covered glass bottle and kept in the dark for approximately 7 days until all the green color had bleached out. Finally, 3.5 ml of the solution was transferred to measure absorbance at two wavelengths using a light spectrophotometer (UV-3101P, Labomed Inc, USA). The two wavelengths of 664nm and 647nm were used as the peak absorbances of chlorophyll-a and chlorophyll-b. The total amount of chlorophyll a and chlorophyll b are then calculated according to the method of Sheikh et al., [37]. Observations did every month after treatments.

Chlorophyll a content (mg/cm$^2$ fresh leaf) = 13.19 ($A_{664}$) - 2.57 ($A_{647}$) 

Chlorophyll b content (mg/cm$^2$ fresh leaf) = 22.1 ($A_{647}$) - 5.26 ($A_{664}$)

Total Chlorophyll content (mg/cm$^2$ fresh leaf) = $\frac{3.5 x (\text{Chl}~a+\text{Chl}~b)}{4}$

Where: $A_{647}$ and $A_{664}$ represent absorbance of the solution at 647 and 664 nm, respectively, while 13.19, 2.57, 22.1 and 5.26 are the absorption coefficients, 3.5 was the total volume used in the analysis taken from the original solution (mL) and 4 was the total discs area (cm$^2$).

2.4. Statistical analysis

Regression and correlation analyses were done with Excel 2013 (Microsoft, USA) and using Statistic Analysis System (SAS) version 9.4.

3. Results

3.1. SPAD value and chlorophyll content

Fig. 1 and Fig.2 showed the relationships between the SPAD-502 value and chlorophyll content of fig leaves at IBT and MD varieties. With increase of SPAD values, the chlorophyll content showed a trend of synchronous decrease. At IBT variety, an exponential mathematical model was fitted best in relationships between total chlorophyll content and SPAD value. Meanwhile, at MD variety, a polynomial 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 fig leaf, content of chlorophyll a was much higher than chlorophyll b and it also correlated better with SPAD value than chlorophyll b.

3.2. Regression analysis of correlation of SPAD value and chlorophyll content

Table 1 showed different mathematical modelling functions, correlations between SPAD value, as x, chlorophyll content, as y, were significantly different and correlations changed with different categories at IBT variety. For total chlorophyll content and chlorophyll a, the highest correlation occurred both in exponential model function, with $Y=29.738e^{-0.072x}$ (r = 0.5380) and $Y=30.209e^{-0.081x}$ (r = 0.6623) respectively. However, power model function model fitted best to chlorophyll b as $Y=388.42x^{-1.676}$ (r = 0.3145).

Table 2 showed different mathematical modelling functions, correlations between SPAD value, as x, chlorophyll content, as y, were also significantly different at MD variety. Correlations between SPAD value and chlorophyll b were generally lower than two other categories. Different at variety, highest correlation for chlorophyll a occurred in exponential model function, with $Y=7.452e^{-0.032x}$ (r = 0.5318). Whilst the fitted best correlation for chlorophyll b and total chlorophyll content occurred at polynomial model function, with $y=-0.0025x^2+0.1268x+0.2896$ (r = 0.3407) and $Y=-0.0052x^2+0.1961x+3.1882$ (r = 0.5129), respectively.
Figure 1. Correlation of SPAD value and chlorophyll content in IBT fig leaves

Figure 2. Correlation of SPAD value and chlorophyll content in MD fig leaves
Table 1. Regression analysis of correlations SPAD value with several mathematic models of chlorophyll content on IBT variety

| Category | linear model, \( y = ax+b \) | Exponential Model, \( \text{Y} = ae^{bx} \) | logarithmic model, \( y = a \ln (x)+ b \) | Polynomial Model, \( \text{Y} = ax^2+bx+c \) | Power model, \( y = ax^b \) |
|----------|------------------|------------------|------------------|------------------|------------------|
| Chl a    | \( Y=-0.1993x+8.6507 \) | \( Y=30.209e^{-0.081x} \) | \( Y=-6.176ln(x)+23.673 \) | \( Y=-0.0114x^2+0.5127x-2.4105 \) | \( Y=13519x^{-2.511} \) |
|          | \( R^2 = 0.3582 \) | \( R^2 = 0.4386 \) | \( R^2 = 0.3560 \) | \( R^2 = 0.3614 \) | \( R^2 = 0.4343 \) |
|          | \( r = 0.5985 \) | \( r = 0.6623^* \) | \( r = 0.5967^* \) | \( r = 0.6012^* \) | \( r = 0.6590^* \) |
| Chl b    | \( Y=-0.0827x+3.8247 \) | \( Y=6.4968e^{-0.054x} \) | \( Y=-2.583ln(x)+10.127 \) | \( Y=-0.0117x^2-0.8102x+15.126 \) | \( Y=388.42x^{-1.676} \) |
|          | \( R^2 = 0.073 \) | \( R^2 = 0.0977 \) | \( R^2 = 0.0738 \) | \( R^2 = 0.0771 \) | \( R^2 = 0.0989 \) |
|          | \( r = 0.2702^* \) | \( r = 0.3126^* \) | \( r = 0.2717^* \) | \( r = 0.2777^* \) | \( r = 0.3145^* \) |
| T Chl    | \( Y=-0.2467x+10.916 \) | \( Y=29.738e^{-0.072x} \) | \( Y=-7.664ln(x)+29.575 \) | \( Y=0.0002x^2-0.2603x+11.126 \) | \( Y=667.7x^{-2.225} \) |
|          | \( R^2 = 0.2127 \) | \( R^2 = 0.2894 \) | \( R^2 = 0.2124 \) | \( R^2 = 0.2127 \) | \( R^2 = 0.2883 \) |
|          | \( r = 0.4612^* \) | \( r = 0.5380^* \) | \( r = 0.4609^* \) | \( r = 0.4612^* \) | \( r = 0.5369^* \) |

* means significant difference p < 0.05.

Table 2. Regression analysis of correlations SPAD value with several mathematic models of chlorophyll content on MD variety

| Category | linear model, \( y = ax+b \) | Exponential Model, \( \text{Y} = ae^{bx} \) | logarithmic model, \( y = a \ln (x)+ b \) | Polynomial Model, \( \text{Y} = ax^2+bx+c \) | Power model, \( y = ax^b \) |
|----------|------------------|------------------|------------------|------------------|------------------|
| Chl a    | \( Y=-0.089x+5.7023 \) | \( Y=7.4524e^{-0.032x} \) | \( Y=-2.122ln(x)+10.208 \) | \( Y=-0.0035x^2+0.0974x+3.3541 \) | \( Y=36.63x^{-0.756} \) |
|          | \( R^2 = 0.2511 \) | \( R^2 = 0.2828 \) | \( R^2 = 0.2262 \) | \( R^2 = 0.2761 \) | \( R^2 = 0.248 \) |
|          | \( r = 0.5011^* \) | \( r = 0.5318^* \) | \( r = 0.4756^* \) | \( r = 0.5255^* \) | \( r = 0.4980^* \) |
| Chl b    | \( Y=0.0051x+1.9514 \) | \( Y=1.9692e^{-0.048x} \) | \( Y=-0.058ln(x)+1.9948 \) | \( Y=-0.0025x^2+0.1268x+0.2896 \) | \( Y=2.1273x^{-0.055} \) |
|          | \( R^2 = 0.0072 \) | \( R^2 = 0.0149 \) | \( R^2 = 0.0015 \) | \( R^2 = 0.1161 \) | \( R^2 = 0.0054 \) |
|          | \( r = 0.0849^* \) | \( r = 0.1221^* \) | \( r = 0.0387^* \) | \( r = 0.3407^* \) | \( r = 0.0735^* \) |
| T Chl    | \( Y=-0.0823x+6.697 \) | \( Y=7.7488e^{-0.021x} \) | \( Y=-1.907ln(x)+10.677 \) | \( Y=-0.0052x^2+0.1961x+3.1882 \) | \( Y=21.542x^{-0.491} \) |
|          | \( R^2 = 0.2091 \) | \( R^2 = 0.249 \) | \( R^2 = 0.1778 \) | \( R^2 = 0.2634 \) | \( R^2 = 0.2095 \) |
|          | \( r = 0.4573^* \) | \( r = 0.4990^* \) | \( r = 0.4217^* \) | \( r = 0.5129^* \) | \( r = 0.4577^* \) |

* means significant difference p < 0.05.

4. Discussions
The regression coefficient between SPAD value and three chlorophyll parameters was highest in total chlorophyll content, both in IBT and MD varieties (Table 1 and 2). Since the wavelength for the leaf transmittance measurement in SPAD-502 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 IBT variety, correlation of \( r \) in the optimal function was 23.10% higher than \( r \) of chlorophyll a and 42.90% higher than \( r \) of total chlorophyll. In contrast, correlation of \( r \) in the optimal function was 35.94% lower than \( r \) of chlorophyll a and 3.55% lower than \( r \) of total chlorophyll respectively. Given the fact that leaf condition would affect the accuracy of correlation analysis [24, 25,26], 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 [38].

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 [10, 22, 36, 39, 40, 41]. These results are different to ours, which suggests that fig 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.

5. Conclusions
There was a significant relationship between SPAD-502 value and chlorophyll content in fig leaves. But the optimized mathematical model for estimation chlorophyll content with SPAD value of leaves on two fig cultivars were different. With the highest correlation efficiency of five mathematic modelling functions at two fig varieties, the results demonstrated that optimal model for chlorophyll a was exponential function both in IBT and MD variety, which was opposite to chlorophyll b was power function at IBT variety and polynomial function at MD variety. Moreover, for total chlorophyll content was exponential function at IBT variety and polynomial function at MD variety.

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