

# 7. Detection of Chlorophyll Content Based on Spectral Properties at Leaf Level: A Meta-Analysis

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# Detection of Chlorophyll Content Based on Spectral Properties at Leaf Level: A Meta-Analysis

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**Abstract**—In accordance to agriculture 4.0, sensor plays an important role for sensing the nutrition status in plants. Generally, chlorophyll meter or spectral sensor is used to measure chlorophyll content to identify plant nutrient deficiencies. The objective of this paper is to obtain correlation coefficient value that indicate the relationship or correlation among chlorophyll measurement using spectrophotometer, chlorophyll meter, and spectral sensor. Here, we thoroughly focus on a meta-analysis of the chlorophyll measurement using 42 papers as a main study. Nevertheless, 12 studies were ejected because of incomplete statistical information. Finally, 5 articles were used in a meta-analysis. The results showed significant correlations among spectrophotometer values, SPAD-502 values, CCM-200 values, atLeaf+ values, and TCS230 sensor values.

**Keywords**—chlorophyll meter, meta-analysis, leaf level, spectral properties

## I. INTRODUCTION

Information on plant nutrition needs is very important for the process of fertilizing plants. Nitrogen, the basic component in fertilizer could also be monitored based on the chlorophyll measurement, as the nitrogen supply and chlorophyll content are highly correlated [1]. Spectrophotometer is usually used to analyze chlorophyll pigment on plant leaves using light absorption by the extracted pigment, however this method has the disadvantage such as laborious, time-consuming and destructive [2].

With the advancement of sensor technology, the ability of leaves to absorb light allows detection of chlorophyll content. This detection is based on the processing reflected and / or transmitted light by the leaves. Chlorophyll meter is a device used to measure chlorophyll content at the leaf level. Chlorophyll meters using visible light sources, infrared light sources and spectral sensors as light detectors. Chlorophyll meter and spectral sensor has been widely used in many studies for chlorophyll content measurement of various type of plants. However, reports on meta-analysis of the chlorophyll concentration measurement using spectral sensor, chlorophyll meter and spectrophotometer are very limited. Therefore, in this paper, we focused on a meta-analysis of chlorophyll detection based on spectral properties at leaf level. Data was extracted from a wide selection of studies which measured chlorophyll concentration using chlorophyll meter, spectral sensor and spectrophotometer

## II. LITERATURE STUDY

### A. Chlorophyll meters

Currently, various chlorophyll meters have been produced with different modes, wavelengths and sensor types. The existing chlorophyll meters are either using transmittance mode such as: SPAD-502, TYSA, MC100, CCM-200, atLeaf+, CL-01, and Dualex (Table I) or using reflectance mode such as CM-1000, Green Seeker and Yara N-Sensor [2].

L. Murdock et al [3] have compared chlorophyll content measurement on wheat plants using two types of chlorophyll meters such as SPAD-502 and CM1000. The results have shown that both meters have good performance and respond in the same way. SPAD-502 uses a closed room and measures the light transmitted by a leaf. Another type of chlorophyll meter such as CM1000 which measures ambient light and reflection is used to calculate relative chlorophyll index. These tools which using transmittance mode are effective in agricultural land as they are not interfered by the sunlight. However, they could only measure one spot from one leaf for each measurement, hence they require big sample of measurements in order to get a reliable data. The reflection mode, on the other hand are easy and fast to use. Though, this mode are depends on sunlight, limited by the position of the sun and cloudy weather conditions, and the angular position affects the measurement results.

TABLE I. COMPARISON OF CHLOROPHYLL METER PRODUCTS BASED ON TRANSMITTANCE MODE

	SPAD-502	CCM-200	MC-100	atLeaf+
Scale	-9.0 to 199.9	0 to 99.9	0 to 99.9	0 to 99.9
Unit	SPAD	CCI	$\mu\text{mol/m}^2$ , SPAD & CCI (optional)	CCI
Accuracy	$\pm 1.0$	$\pm 1.0$	$\pm 10$ ( $\mu\text{mol/m}^2$ ) $\pm 1.0$ (CCI)	$\pm 1.0$
Reading time	< 2 seconds	2 - 3 seconds	< 3 seconds	< 1 seconds
Wavelength	650 nm 940 nm	653 nm 931 nm	653 nm 931 nm	640 nm 940 nm
Light source	RED & IR	RED & IR	RED & IR	RED & IR
Sensor	Silicon photodiode	Silicon photodiode with internal amplifier	Silicon semiconductor photodiode	Silicon semiconductor photodiode

### B. Effect size calculation

The strength of the relationship between chlorophyll concentration measurement using spectral sensor, chlorophyll meter and spectrophotometer in each article is evaluated using the coefficient of determination ( $R^2$ ) [4]. In general, synthesis on meta-analysis is not carried out using correlation coefficients. Correlation coefficients are converted to Fisher  $z$  scale, and these values are used in all analyzes. The analysis results are then converted back to correlation [5]. Conversion from sample correlation  $r$  to Fisher's  $z$  is calculated using:

$$z = 0.5 \times \ln \left( \frac{1+r}{1-r} \right) \quad (1)$$

The variance of  $z$  is calculated using:

$$V_z = \frac{1}{n-3} \quad (2)$$

and the standard error is calculated using:

$$SE_z = \sqrt{V_z} \quad (3)$$

The weight in the study was calculated as:

$$W = \frac{1}{V_z} \quad (4)$$

$$W \% = \frac{W}{\sum W} \times 100\% \quad (5)$$

### C. Heterogeneity test for effect sizes

Before calculating the average effect size, heterogeneity between research results is important to be assessed. The percentage of heterogeneous components in the total variability of effect size ( $Q_{tot}$ ) is defined as  $I^2$ .  $T^2$  is defined as variation among studies.  $I^2$ , dan  $T^2$  is calculated using [4]:

$$I^2 = \frac{Q_{tot} - df}{Q_{tot}} \quad (6)$$

$$T^2 = \frac{Q_{tot} - df}{C_{tot}} \quad (7)$$

Where  $df = N_{tot} - 1$ , the total number of the selected studies ( $N_{tot}$ ).  $Q_{tot}$  and  $C_{tot}$  is calculated as [4]:

$$Q_{tot} = \sum_{i=1}^{N_{tot}} W_i \cdot (Z_i)^2 - \frac{(\sum_{i=1}^{N_{tot}} W_i Z_i)^2}{\sum_{i=1}^{N_{tot}} W_i} \quad (8)$$

$$C_{tot} = \frac{W_{tot} - (W_{tot})^2}{W_{tot}} \quad (9)$$

The stronger the heterogeneity, the greater the  $I^2$ . If  $I^2$  is greater than 0.5, the null hypothesis of homogeneity is rejected [4]. If  $I^2$  is lower 0.5, the null hypothesis of homogeneity for this study is accepted. If the  $p$  value of heterogeneity test  $> 0.05$  or a small  $I^2$  value, this indicates that the inter-research does not vary therefore to calculate the combined effect using the fixed effects model. Combined effect with Fixed effect model calculated as [6]:

$$EG = \frac{\sum W \cdot z}{\sum W} \quad (10)$$

If the  $p$  value of the heterogeneity test is  $< 0.05$  or a large  $I^2$  value, this indicates that the inter-research varies therefore random effects model is used to calculate the combined effect. Total variation ( $V_t$ ) and the total research weight ( $W_t$ ) values are needed to calculate the combined effect with the random effect model. Total variation ( $V_t$ ) is calculated as [6]:

$$V_t = V_z + T^2 \quad (11)$$

The total research weight ( $W_t$ ) is calculated as [6]:

$$W_t = \frac{1}{V_t} \quad (12)$$

$$W_i \% = \frac{W_i}{\sum W_i} \times 100\% \quad (13)$$

Combined effects with the Random effect model are calculated as [6]:

$$EG_r = \frac{\sum W_i z}{\sum W_i} \quad (14)$$

After the combined effect is obtained, then the value is converted to the correlation unit using the formula below:

$$r = \frac{e^{2EG_r} - 1}{e^{2EG_r} + 1} \quad (15)$$

The results of this meta-analysis are combined effects consist of the value of the weight of each study, correlation coefficient and confidence interval.

## III. METHODS

Meta-analysis relates to a series of procedures for analyzing the coefficients reported in published studies. This method allows researchers to collect findings from several studies to draw valid conclusions [4]. We have been conducted meta-analysis with research stages consisting of 7 steps as follows [7].

- Step 1: Formulating the problem
- Step 2: Searching the literature
- Step 3: Gathering information from studies
- Step 4: Evaluating the quality of studies
- Step 5: Analyzing and integrating the outcomes of studies
- Step 6: Interpreting the evidence
- Step 7: Presenting the results

In this paper, meta-analysis research aimed to provide answers to several research questions, such as:

- What is chlorophyll meter mode widely used?
- What is chlorophyll meter widely used?
- What are wavelength area used in chlorophyll meter?
- What are the strength of the relationships or correlation among chlorophyll measurement using spectrophotometer, chlorophyll meter, and spectral sensor?

Database of ACM Digital Library, SCOPUS, Science Direct, Springer Link, IEEE Xplore Digital Library, and

EBSCOhost were used for source data. The research paper was published in 1986 to 2017, and the following key words were used such as chlorophyll meter, nitrogen, and wavelength. The results of this initial search more than 243 citations were collected from database search. Then the abstracts of these studies were reviewed and considered for inclusion in the meta-analysis. The studies were reviewed based on the following criteria. First, the studies had to include a measurement of chlorophyll concentration (total chlorophyll) using chlorophyll meter and/or spectrophotometer. Second, the research paper provided statistical information such as coefficient determination or coefficient correlation and number of sample. Based on the first criteria, 42 papers were selected, and 12 papers were excluded. Finally, 5 articles were used in meta-analysis and 36 studies were excluded. Fig 1 shows the Stages of obtaining papers as meta-analysis material.

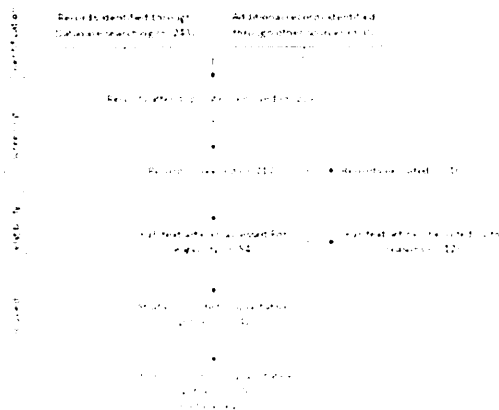


Fig 1. Stages of obtaining papers as meta-analysis material

#### IV. RESULT

Relevant information was extracted from each study in the final set such as chlorophyll meter or spectral sensor, mode, year, references, and species. the studies used in this study (Table II) [8].

TABLE II. THE STUDIES USED IN THIS STUDY

Chl. Meter/ Mode	Year	References	Species
SPAD-501/ Transmittance	1986	[9]	22 Unrelated species
	1987	[10]	12 Unrelated species
	1991	[11]	8 Tropical and sub tropical fruit tree species
	1991	[12]	Maize
	1991	[13]	12 Wine grape cultivars
SPAD-502/ Transmittance	1992	[14]	6 Sclerophytious species
	1992	[15]	Wheat, soybean, rice
	1995	[16]	Maize and soybean
	2000	[17]	Sorghum
	2002	[18]	Potato
	2002	[19]	Papaya

	2002	[20]	Sorghum and pigeonpea
	2003	[21]	4 Citrus species
	2004	[22]	Peac lily
	2005	[23]	Coffee
	2005	[24]	Wheat
	2007	[25]	Birth, wheat, and potato
	2008	[26]	Corn
	2008	[27]	Cassava
	2010	[28]	Flowering cherry
	2010	[29]	13 Tree species of tropical rain forest
	2010	[30]	<i>Eugenia uniflora</i>
	2011	[31]	<i>Arabidopsis thaliana</i>
	2014	[32]	Oat
	2014	[33]	<i>Andropogon gerardii</i> (Poaceae)
	2016	[34]	<i>Eucalyptus dunnii</i>
	2017	[35]	Cucumber
Yara N-Sensor & GreenSeeker/ Reflectance	2009	[36]	Wheat, corn
CM-1000/ Reflectance	2014	[37]	Grass (Fescue)
CCM-200/ Transmittance	2004	[38]	Sugar maple
	2008	[39]	4 Tropical wood species from Amazonian forest
	2010	[40]	Quercus
	2014	[8]	22 Species
SPAD-502 and all.EAF +/- Transmittance	2012	[41]	6 Crop species (potato, barley, canola, wheat, and corn)
	2016	[2]	<i>Cleistanthus calamus</i>
	2016	[42]	Lettuce, leaf Mustard, radish and cabbage
SPAD-502 and CCM-200/ Transmittance	2002	[43]	Paper birth
	2005	[44]	6 Citrus species
	2012	[45]	Kiwi, grape, wheat and maize
SPAD-502 / Transmittance and CM-1000/ Reflectance	2004	[3]	Wheat
TCS230 sensor and SPAD-502	2012	[1]	Lettuce
	2017	[46]	Cassava

The highest number of research publications occurred in 2002, 2010 and 2014 (Fig 2). Most of the study using chlorophyll meter based on the transmittance mode (Fig 3) and most of the study using SPAD-502 (Fig 4).



Fig 2. The number of research publications on the measurement of chlorophyll during the last 20 years

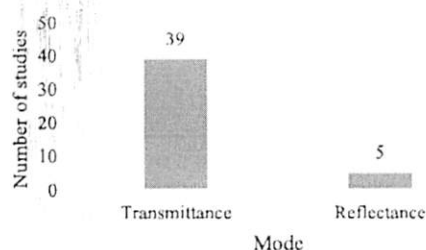


Fig 3. The number of research publications using chlorophyll meter based on transmittance and reflectance modes

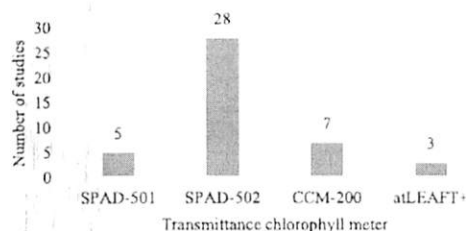


Fig 4. The number of research publications using chlorophyll meter based on transmittance mode

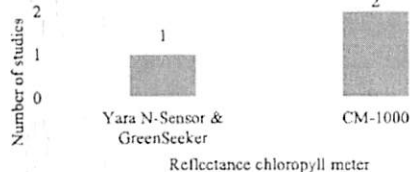


Fig 5. Number of research publications using chlorophyll meter based on reflectance mode

Table III shows that transmittance mode used the wavelength ranging from 620 nm to 660 nm for light absorbed by chlorophyll and 850 nm to 940 nm for light that is not absorbed by chlorophyll. Meanwhile the reflectance mode measure chlorophyll content by using wavelength between 450 nm and 900 nm (Table IV).

TABLE III. THE WAVELENGTH AREA USED IN CHLOROPHYLL METERS BASED ON TRANSMITTANCE MODE.

Chlorophyll meter	Wavelength
SPAD-502 and TYSA	650 nm and 940 nm
MC100 and CCM-200	653 nm and 931 nm
atLEAF+	660 nm and 940 nm
CL-01	620 nm and 940 nm
Duallex	710 nm and 850 nm

TABLE IV. THE WAVELENGTH AREA USED IN CHLOROPHYLL METERS IS BASED ON TRANSMITTANCE MODE.

Chlorophyll meter	Wavelength
CM-1000	700 nm and 840 nm
Green Seeker	656 nm and 774 nm
Yara N-Sensor	450 nm and 900 nm

The results of heterogeneity test for effect sizes shows the value of  $p = 0.00$  and  $I^2 = 0.875618$  (table V), the value of  $p = 0.00$  and  $I^2 = 0.896122$  (table VI), thereafter the calculation of the combined effect uses random effect model. Meta-analysis results show correlation coefficient ( $r$ ) = 0.95 and confidence interval between 0.94 to 0.95 (table V); correlation coefficient ( $r$ ) = 0.97 and confidence interval between 0.97 to 0.98 (table VI).

TABLE V. META-ANALYSIS OF CHLOROPHYLL CONCENTRATION MEASUREMENT USING SPAD-502, CCM-200, atLEAF+ COMPARED TO SPECTROPHOTOMETER AS COMPARISON

Study	Species	Statistics for each study		Random Effect Model			
		$n$	$r^2$	$w$	$r$	$min$	$max$
[29]	<i>Amanoa guianensis</i>	30	0.98	0.6	0.95	0.94	0.95
[29]	<i>Bagassa guianensis</i>	30	0.97	0.6			
[29]	<i>Carapa procera</i>	30	0.98	3.8			
[29]	<i>Cecropia obtusa</i>	29	0.92	3.7			
[29]	<i>Eperua falcata</i>	32	0.78	4.1			
[29]	<i>Hymenaea courbaril</i>	34	0.91	4.4			
[29]	<i>Inga thibaudiana</i>	34	0.94	4.4			
[29]	<i>Pouteria sp.</i>	28	0.92	3.5			
[29]	<i>Protium opacum</i>	31	0.94	4.0			
[29]	<i>Sextonia rubra</i>	30	0.99	3.8			
[29]	<i>Symphonia globulifera</i>	30	0.98	3.8			
[29]	<i>Tachigali melinonii</i>	23	0.97	2.8			
[29]	<i>Vouacapoua americana</i>	30	0.91	3.8			
[25]	Birch	60	0.93	8.1			
[25]	Wheat	72	0.89	9.8			
[25]	Wheat, Dragon 1999	45	0.81	6			
[25]	Wheat, Lantvete 1999	45	0.85	6			
[38]	Sugar maple	98	0.76	13.5			
[41]	Canola, Wheat,	50	0.78	6.7			

	Barley, Potato, Corn (SPAD- 502)			
[41]	Canola, Wheat, Barley, Potato, Corn (atLeaf+)	50	0.72	6.7
Total Weight				100

TABLE VI. META-ANALYSIS OF CHLOROPHYLL CONCENTRATION MEASUREMENT USING atLeaf+ AND TCS230 SENSOR COMPARED TO SPAD-502 AS COMPARISON

Study	Species	Statistics for each study		Random Effect Model			
		n	r <sup>2</sup>	w	r	min	Max
[2]	Cleistanthus	60	0.84	1.1	0.97	0.97	0.98
[2]	Calamus	60	0.73	1.1			
[41]	Canola	20	0.86	4.3			
[41]	Wheat	20	0.78	4.3			
[41]	Barley	20	0.92	4.3			
[41]	Potato	20	0.90	4.3			
[41]	Corn	20	0.92	4.3			
[1]	Lettuce	10	0.86	1.8			
[46]	Casava	295	0.97	74.4			
Total Weight				100			

## V. CONCLUSION

Chlorophyll meter measures chlorophyll content at the leaf level using transmittance and reflection modes. The most widely used mode for chlorophyll meters is transmittance mode and SPAD-502 is the most widely used chlorophyll meter. Meta-analysis was carried out with two scenarios namely the first meta-analysis of the measurement of chlorophyll content using SPAD-502, CCM-200, atLeaf + compared to spectrophotometers as a comparison, and the second meta-analysis of the measurement of chlorophyll content using atLeaf+ and TCS230 sensors compared to SPAD-502 as a comparison. Results from heterogeneity tests, both meta-analysis indicate heterogeneous research results therefore the analysis model to calculate the combined effect using the random effect model. The results show there were significant correlations between the values of SPAD-502 or CCM-200 or atLEAF+ with spectrophotometer values ( $r=0.95$ ) and there were significant correlations between atLeaf+ values or TCS230 sensor values with SPAD-502 values ( $r=0.97$ ). Statistically the relationship between the measurement results of chlorophyll content using a chlorophyll meter or spectral sensor and spectrophotometer is significant because the confidence interval does not exceed zero.

## VI. FUTURE WORK

In our future work, we will develop a low-cost device to measure chlorophyll content.

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