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Rapid Determination of Leaf Chlorophyll Concentration, Photosynthetic Activity and NK Concentration of *Elaies guineensis* Via Correlated SPAD-502 Chlorophyll Index

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ABSTRACT
Determination of chlorophyll contents often requires destructives analysis and involves lengthy procedures while measurement on photosynthetic rate requires specialized equipment that often limits extensive in-field application. SPAD chlorophyll meter gives indirect measurement of leaf chlorophyll contents in a rapid, non-destructive and convenient manner. The use of SPAD meter for each individual plant species require calibration curve for each individual plant species, as the accuracy of the estimation are highly affected by the leaf anatomical characteristics. This study reports the result of correlating SPAD-502 chlorophyll index readings to the actual extracted chlorophyll content and photosynthetic rate of oil palm leaf as well as the leaf nitrogen and potassium concentration. Concentration of oil palm leaf nitrogen and chlorophyll pigment as well as photosynthetic rate were highly correlated to SPAD-502 chlorophyll index with R^2 of above 0.80. With good calibration equation, the SPAD-502 chlorophyll meter are extremely versatile for rapid determination of oil palm leaf nitrogen and chlorophyll concentration as well as photosynthetic rate.

Key words: Oil palm, SPAD meter, chlorophyll, nitrogen, photosynthetic rate, regression

INTRODUCTION
Plants photosynthetic rate and chlorophyll contents are major factors influencing plant growth and nutritional responses towards experimental inputs. The determination of chlorophyll contents from leaves with chemical solvents is laborious and requires lengthy procedures. In addition, field measurement of photosynthetic rate requires specialized equipment which is tedious to operate and often with limited access in developing countries.

SPAD meter which was originally developed in Japan for nitrogen management in rice (*Oryza sativa*) production (Wood *et al*., 1993) is now commonly used for rapid and non-destructive estimation of foliar chlorophyll concentrations. SPAD-502, the latest model measures the relative greenness of leaves in a rapid manner (Loh *et al*., 2002). The device quantifies the relative amount of chlorophyll present in leaf by measuring the leaf transmittance in two wave bands (400-500 nm and 600-700 nm) and reports the readings in arbitrary unit (SPAD-502 Chlorophyll Index, SCI) that is proportional to the leaf chlorophyll concentration.

Table 1: Calibration models for total chlorophyll contents against SPAD chlorophyll reading of reported species

Plant species	Fitted equation line	R^2	Total chlorophyll (μg g^{-1} fresh weight) at respective SPAD-502 chlorophyll index (SCI) 20	30	50	Reference
Acer pseudoplatanus	$Y = 0.0075X^2+0.998X-0.4916$	0.93	22.5	36.2	68.2	Glynn *et al.* (2008)
Fagus sylvatica	$Y = -0.0029X^2+1.175X+3.9506$	0.85	26.3	36.6	55.5	Glynn *et al.* (2008)
Quercus robur	$Y = 1.8159X^{0.8809}$	0.89	25.4	36.3	57.0	Glynn *et al.* (2008)
Euphorbia pulcherrima	$Y = 2.0673X^{1.4636}$	0.92	165.8	300.1	633.9	Schuch *et al.* (1995)
Plant species	Fitted equation line	R^2	Total chlorophyll (μg g^{-1} dry weight) at respective SPAD-502 chlorophyll index (SCI) 20	30	50	Reference
Ficus benjamina	$Y = 35.6X-298$	0.90	414.0	770.0	1482.0	Loh *et al.* (2002)
Populus deltoides	$Y = 1.65X^2-11.21X+423.19$	0.96	859.0	1571.9	3987.7	Loh *et al.* (2002)

Leaf chlorophyll content are often well correlated with photosynthetic activity and leaf N status (Evans, 1983; Seemann *et al.*, 1987). Due to the excellent correlation of SPAD-502 readings with chlorophyll content and N status of crops and rapid results readiness the SPAD meter is now widely accepted in the agronomy industry (Loh *et al.*, 2002). SPAD-502 meter had been reported accurately to predict the chlorophyll content in rice (Takebe and Yoneyama, 1989), maize (Wood *et al.*, 1993) and wheat (Follett *et al.*, 1992).

Despite the convenience of the SPAD-502 meter in determining chlorophyll concentration, it is not without its own limitation. Several studies have been reported that SPAD-502 meter gave differing prediction responses for different plant species (Marquard and Tipton, 1987; Schaper and Chacko, 1991). Correlation between the SCI and the actual leaf chlorophyll content were species specific. The regression lines correlating chlorophyll content to SCI of several reported species were tabulated in Table 1.

The usability of SPAD meter in a variety of crops requires transformation of the indices generated by the SPAD meter into interpretable data. This is because indices calibration is required as the sensor transform signal into indices that could potentially cause variation before giving any meaningful readings (Marquard and Tipton, 1987). Consequently any effort to generate calibration models requires individual regression models be established for each plant species. Hence the objective of this experiment was to generate a calibration model for associating the SPAD data to several physiological measurements such as leaf chlorophyll concentration, total leaf nitrogen and potassium concentration as well as leaf photosynthetic rate of oil palm (*Elaies guineensis* v. Jacq).

MATERIALS AND METHODS

Plant material: Six months old oil palm (*E. guineensis* v. Jaq) seedlings grown as part of a study on progeny growth responses to potassium supplies (Sim *et al.*, 2013) was used in this study. Oil palm progenies of selected origins were supplied by Applied Agricultural Resources Sdn. Bhd. Three-months old seedlings were transplanted into polybag filled with 25 kg of Serdang series soil (*Typic kandiudults*) and grown for three months before they were used in the experiment. Seedlings were fertilized with standard fertilizer programme adopted by most commercially operated oil palm nursery and grown under natural photo period and irradiance (Tan, 2011).

Measurements of SPAD-502 chlorophyll index and photosynthetic rate: Leaf blades from Frond No. 3 with visually different green colours in order to maximize the calibration range of

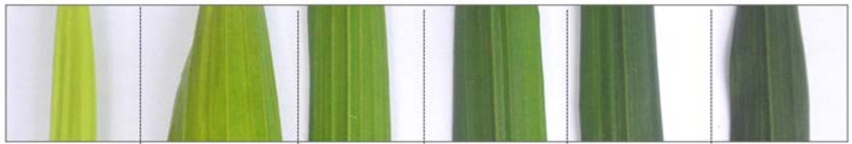

Fig. 1: Variation of leaf colour and SPAD readings selected for this experiment

Table 2: Calculation of chlorophyll a and b as well as total carotenoids

Parameters	Equation proposed
Chlorophyll a	C_a = 12.47×A665.1-3.62×A649.1
Chlorophyll b	C_b = 25.06×A649.1-6.5×A665.1
Carotenoids	C_{x+c} = (1000*A480-1.29×C_a-53.78×C_b)/220

SPAD-502 meter were sampled (Fig. 1). The identified leaf samples were surface wipe cleaned with distilled water and 3 random leaf spots around the midpoint of each leaf blade were marked for SPAD-502 Chlorophyll Index (SCI) and photosynthetic rate measurements. The leaf spots were later sampled and bulked for determination of leaf chlorophyll, nitrogen and potassium concentrations.

Leaf chlorophyll, nitrogen and potassium concentration: Leaf area where the triplicate SCI and photosynthetic readings were obtained, were sampled for chlorophyll contents determination. A total of 40 leaf samples were collected from SCI which varies from 10-70 (Fig. 1).

Conventional paper puncher were used to acquire 6 consistent size leaf disc from each sample. Leaf disc was then submerged in 5 mL of Dimethyl Sulfoxide (DMSO) for chlorophyll extraction according to Hiscox and Israelstam (1979). Samples were first incubated at 70°C for 30 min, cooled and incubated overnight at 4°C. The extracts were assayed using a spectrophotometer at 480, 649 and 665 nm. Chlorophyll a and b contents and carotenoids were determined by following the equations (Table 2) proposed by Wellburn (1994).

The remaining of leaf samples were then dried to a constant weight in an oven at 70°C for 24 h and later processed for N and K determination. Concentrations of K was measured using an AAS while total N was analysed via Kjeldahl method.

Statistical analysis: Data was analyzed and correlated using linear regression procedures of Curve Expert program using quadratic polynomial, logarithmic, exponential or simple linear models. Best fitted model which gave the highest percentage of fitness was determined by using the Curve Expert program.

RESULTS AND DISCUSSION

Relationship between SPAD-502 chlorophyll index with leaf chlorophyll contents: Fitted correlation curves demonstrated that the chlorophyll a and b as well as total carotenoids had quadratic correlation with the SCI (Fig. 2). The regression coefficient, R^2 between chlorophyll contents and SCI ranged from 0.85-0.92 indicating strong relationship between SCI and chlorophyll contents as well as the usability of SPAD-502 meter in predicting the chlorophyll contents in oil palm leaves. Within the extracted chlorophyll pigments, oil palm leaves showed higher amount of chlorophyll a, as 4 times higher than that of chlorophyll b.

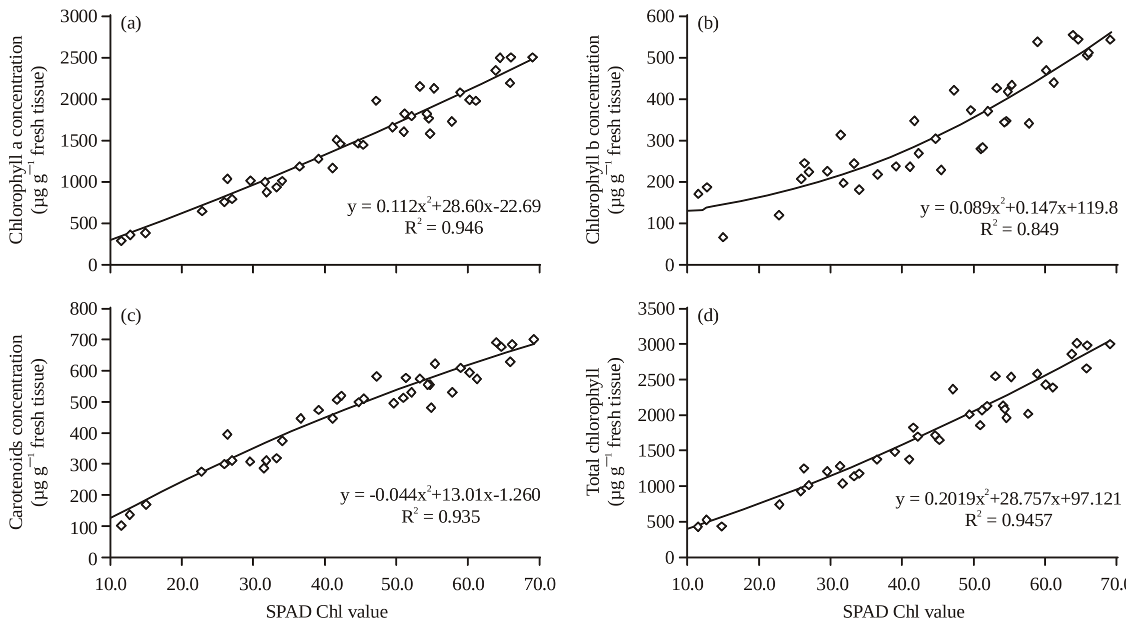

Fig. 2(a-d): Calibration curves for (a) Chlorophyll a, (b) b, (c) Total carotenoids and (d) Total leaf chlorophyll contents of oil palm leaves with SPAD-502 chlorophyll index

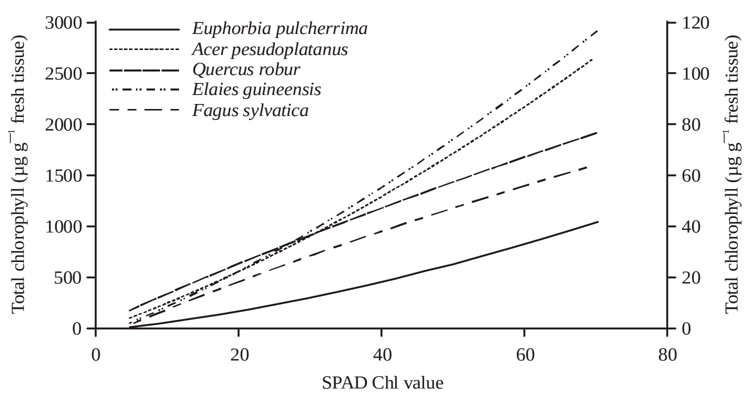

Fig. 3: Comparison of the relationship of total leaf chlorophyll contents versus SPAD-502 chlorophyll index of several plant species *Calibration curves of other plant species were re-plotted from the reported equation in Table 1. Primary Y-axis: *E. guineensis* and *E. pulcherrima*, secondary Y axis: *A. pseudoplatanus*, *F. sylvatica* and *Q. robur*

Comparing reported data collected from other plant species, oil palm leaves contain significantly higher total amount of chlorophyll a and b (Fig. 3). Within the same unit of SCI value, the chlorophyll concentration in oil palm were approximately 5 times higher than *E. pulcherrima*. This further validate the need of developing specific correlation equation for cross species interpretation of SPAD-502 readings. High R^2 achieved further confirmed the robustness for use in other plant species.

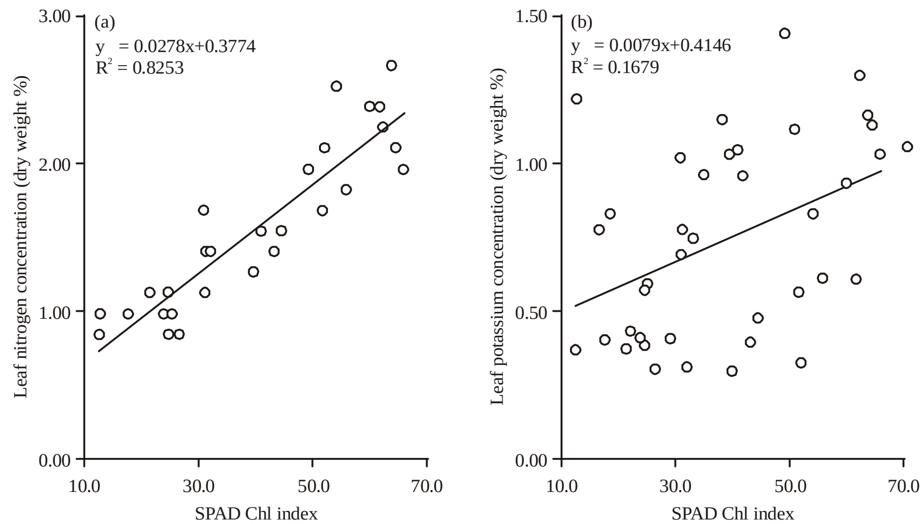

Fig. 4(a-b): Correlation of oil palm leaf (a) Nitrogen and (b) Potassium concentration to SPAD-502 chlorophyll index

Such variation is much expected as SPAD reading is affected by the leaf anatomical characteristics such as leaf thickness, fresh and dry leaf mass per area, leaf water contents and cuticle reflectance. The leaf anatomical characteristics often are unique to each plant species, hence they explain the variation in SPAD reading and further justify for the need of calibration to actual chlorophyll contents for a reliable and interpretable data. In addition, the choice of wavelength potentially affects the detection accuracy, which the wavelength with large absorption coefficient increase the accuracy at low leaf chlorophyll contents and looses it accuracy at high leaf chlorophyll contents.

Relationship of SCI with leaf nitrogen and potassium concentration: Oil palm leaf nitrogen concentration were linearly correlated with SCI and has an R^2 of 0.83. Intriguingly Law *et al.* (2014) reported similar linear model (Y = 0.027X+0.732) in relationship between oil palm leaf nitrogen concentration and SCI. Results obtained by this experiment (Y = 0.0278X+0.377) and as reported by Law *et al.* (2014) reaffirmed that the coefficient for SCI is between 0.027 and 0.028 as shown in Fig. 4. The coefficient indicates that for every additional 1 SCI unit, the total nitrogen concentration in oil palm leaf would increase by an average of approximately 0.03%. Correlation between leaf potassium concentration and SCI was poor as potassium is not the constituent of the photosynthetic pigments. Hence the usage of SPAD-502 meter for rapid in-field determination of potassium concentration is not recommended.

Relationship between SCI with photosynthetic activity: Photosynthetic rate of oil palm leaf correlated positively to SCI following a logarithmic model with R^2 of 0.82 (Fig. 5). Photosynthetic rate are affected by the chlorophyll concentration. As the chlorophyll concentration correlates well to SCI, hence the photosynthetic rate can be expected to correlate well with SCI. Good correlation between photosynthesis rate and SCI increases the robustness of SPAD-502 meter for non-destructive and rapid determination of photosynthetic rate in the field, thus minimizing the

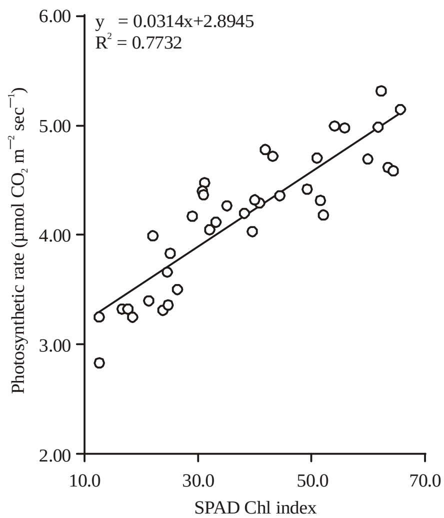

Fig. 5: Logarithmic correlation between photosynthetic rate of oil palm leaf and SPAD-502 chlorophyll index

need of bulky equipment such as the photosynthetic meter. Total replacement of photosynthetic meter is not possible as there are other additional parameters required for further study of the photosynthesis process.

CONCLUSION

Cross species interpretation and comparison of the SPAD-502 Chlorophyll Index (SCI) are not advisable as the readings generated by SPAD-502 meter are affected by leaf structure. Re-establishing the SCI data into expressive readings such as actual chlorophyll concentration is necessary for results interpretation. Oil palm leaf nitrogen concentration, chlorophyll concentration and photosynthetic rate were highly correlated with R^2 of above 0.80. With proper calibration equation, the SPAD-502 chlorophyll meter are extremely versatile for rapid determination of oil palm leaf nitrogen and chlorophyll concentration as well as photosynthetic rate. Correlation between leaf potassium concentration and SCI were poor as potassium is not the constituent of the photosynthetic pigments. Hence the usage of SPAD-502 meter for rapid in-field determination of potassium concentration is not recommended.

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