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Non-destructive determinations of chlorophyll concentration and specific leaf weight using chlorophyll meter during leaf ontogeny in selected fruit species

(Penentuan kepekatan klorofil dan berat spesifik daun melalui kaedah tanpa pemusnahan tisu menggunakan meter klorofil semasa perubahan ontogeni daun untuk spesies buah-buahan terpilih)

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Key words: SPAD reading, chlorophyll concentration, specific leaf weight, fruit trees

Abstract

The chlorophyll meter (SPAD 502) was used to estimate leaf chlorophyll concentration and specific leaf weight of selected tropical fruit species. The objective of this study was to determine the relationships between SPAD readings and leaf chlorophyll concentration and specific leaf weight (SLW) during leaf ontogeny of selected fruit species. Significant correlations (p <0.001) were found for both relationships between SPAD values and dimethylsulfoxide-extracted leaf chlorophyll concentrations and specific leaf weight. Correlation coefficients for the linear regressions of these SPAD values against leaf chlorophyll concentrations were 0.93, 0.93, 0.9, 0.93, 0.88, and 0.71, for banana, carambola, dokong, durian, mangosteen and rambutan respectively. Correlation coefficients for the linear regressions of these SPAD values against SLW were 0.89, 0.84, 0.63, 0.79, 0.72, and 0.78 for banana, carambola, dokong, durian, mangosteen and rambutan respectively. Regression equations for predicting chlorophyll concentrations and specific leaf weight non-destructively were developed for each species. The potential use of chlorophyll meter for leaf physiological indicator was also discussed.

Introduction

Chlorophyll meter (SPAD 502, Minolta Corp.) has been widely used in many important parameters in physiological and agronomic studies on a variety of crop species. It has been successfully used in estimating degree of greenness or chlorophyll contents (Fanizza et al. 1991; Schaper and Chacko 1991; Thompson et al. 1996), a rapid estimate of leaf absorptance in the field (Earl and Tollenaar 1997) and leaf carbon isotope discrimination (Araus et al. 1997). In recent years, the chlorophyll meter has been used as a diagnostic tool to assess nitrogen status of leaves in a variety of annual crops (Fox et al. 1994; Peng et al. 1996; Ma and Dwyer 1997) and perennial crops such as apple (Neilsen et al. 1995).

Leaf growth is an important component in the productivity measurement. Changes in growth with respect to anatomy, morphology and compositions during leaf ontogeny may substantially contribute to the respective changes in the course of leaf photosynthesis

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and related processes (Ticha 1985; Ticha et al. 1985). Changes in physiological activities during the development of individual leaves are usually characterised by an increase to a maximum of activities followed by a decline.

Specific leaf weight (SLW) is the ratio of leaf mass to leaf area. It represents the relative use of carbon for leaf thickening and leaf expansion (Ticha et al. 1985). It is inversely proportionate to specific leaf area which represents the average leaf area expansion per unit dry weight. Besides an important index in leaf structure, SLW is also related to leaf thickness (Chiariello et al. 1989), which is also used as a selection criterion for leaf photosynthesis due to its close relationship with mean photosynthetic rates in many genotypes and species (Ticha et al. 1985).

Specific leaf weight in general is more sensitive to environmental change and therefore more prone to ontogenetic drift (Hunt 1982). Due to its variation among individual species and its plasticity behaviour, many physiologists consider SLW as an indicator for growth performance of a species or as a measure of relative response to various environmental factors (i.e. irradiance and temperature) or resource availability (i.e. nitrogen). The review on variability of SLW in relation to genotype and various environmental factors were well documented (Dijkstra 1990). In many physiological based growth models, the value of SLW or specific leaf area at various developmental stages is needed in the computation of the production of new leaves as well as leaf area index (Driessen and Konijn 1992).

Less attention has been given to the relationship of SPAD values with morphological or physiological activities during leaf ontogeny of tropical fruit species. Ability to estimate these activities without destructive measurements could provide fast and reliable ways in the choice of leaf samples to be used for comparative studies across treatments. It can also be used to monitor growth rate of certain activities during leaf ontogeny in response to certain treatments (i.e. fertiliser, irrigation). Therefore, the purpose of this study was to relate changes in SLW and chlorophyll concentration during leaf ontogeny under field condition as reflected by the chlorophyll meter readings. Based on this relationship, SPAD meter will be used as a tool to estimate chlorophyll content and SLW in selected crop species under field condition.

Materials and methods

Experimental materials

Six popular fruit species grown in Dusun Teknologi, MARDI's Experimental Station in Serdang were used in the experiment. The fruit species were dokong *(Lansium domesticum)*, banana *(Musa paradisiaca)*, carambola (*Averrhoa carambola*, clone B10), durian (*Durio zibethinus*, clone D24), rambutan (*Nephelium lappaceum,* clone R156) and mangosteen (*Garcinia mangostana*, L). All the fruit trees aged between 10 and 15 years old. The fruit species were well maintained under standard agronomic practices. For banana crop, the Berangan cultivar planted in June 2001 was used. Samples were obtained from crop plant (first generation). Number of trees used ranged from 5–10 while leaf samples ranged from 26–42 for each species. Leaf samples of various sizes and colours were collected to represent various ages and stages of leaf ontogeny.

Chlorophyll meter

In recent years, portable field instruments to measure the absorbance of light by the intact leaf were introduced. This non-destructive optical method is dependent on the amount of light absorbed by various photosynthetic pigments in the leaf. A chlorophyll meter model SPAD-502, [Soil-Plant Analysis Development (SPAD) Section, Minolta-Camera Co., Osaka Japan] was used to obtain SPAD values of intact leaves. The SPAD values or readings displayed by this instrument are calculated index to indicate relative amount of chlorophyll contained in plant leaves. The chlorophyll meter calculates the SPAD value based on the intensities of light transmitted in the red band (around 650 nm) where absorption by chlorophyll is high and in the infrared band (around 940 nm) where absorption is low (Minolta Camera 1989). Six chlorophyll meter readings, with three on each side (top, middle and basal) of the midrib were taken from each leaf blade. However, only the average value was used to represent the mean SPAD reading for each leaf. Samples were selected with SPAD values evenly distributed across a broad range, in order to allow regression analysis of the relationships between SPAD reading and leaf chlorophyll concentration and SLW to be established.

Specific leaf weight (SLW)

Leaf areas were measured using area meter LI-3100 (Li-Cor, Lincoln, NE. USA). Leaf dry weight was determined after oven-drying at 70 ºC for 48 h. Specific leaf weight was calculated as the ratio of dry weight to leaf area.

Chlorophyll extraction and determinations

Chlorophyll was extracted using a method of Hiscox and Israelstam (1979). Leaf samples were cut into small pieces and placed in a test tube containing 15 ml Dimethyl sulphoxide (DMSO) and incubated overnight at 65 ºC. Preliminary observations on these species indicated that overnight incubation gave maximum extraction of chlorophyll. A 3.0 ml chlorophyll extract was transferred to a cuvette, and absorbance readings were taken at 645, 663 and 710 nm using Shimadzu Digital Double-Beam Spectrophotometer Model UV-210A (Japan). Absorbance reading at 710 nm was used as an isobestic point (theoretical base line) and was deducted from absorbance readings of 645 nm and 663 nm from the same solution. This procedure was necessary to standardise absorbance readings from various tissues and species used in this study (Hendry and Price 1993). Chlorophyll concentrations of a, b and total were calculated based on MacKinney's equations (MacKinney 1941).

Statistical analysis

Data were subjected to simple linear regression (SAS Inst. 1985), with total chlorophyll concentrations and specific leaf weight as dependent variables and SPAD reading as the independent variable. For all experiments, regression analysis was performed using SAS procedures (SAS Inst. 1985).

Results

Grouping of SPAD readings

In order to make species comparison easier, all SPAD readings for each crop were ranked from low to high into four groups: ≤ 20, 21–35, 36–55 and ≥ 55 *(Tables 1–2)*. These ranges of SPAD values were arbitrarily chosen, however these values did correspond to a broader range of leaf ontogeny to certain extent. Observed values of chlorophyll concentration and SLW within each SPAD group were compared among species under study.

Table 1. The actual chlorophyll concentration based on SPAD range in selected fruit species

Range of SPAD readings	Actual chlorophyll concentration (mg chlorophyll/g tissue fresh weight)					
	Banana	Carambola	Dokong	Durian	Mangosteen	Rambutan
≤ 20	0.42 – 0.44	0.73 – 0.83	0.12 – 0.42	0.14 – 0.54	0.14 – 0.3	0.64 – 1.31
21–35	0.58 – 1.19	0.83 – 1.35	0.51 – 0.87	0.62 – 0.97	0.30 – 0.58	1.23 – 2.06
36–55	0.96 – 2.12	1.33 – 2.01	0.93 – 1.42	0.93 – 1.81	0.51 – 0.89	1.94 – 2.85
≥ 55	1.72 – 2.73	2.06 – 2.54	1.59 – 2.63	1.62 – 2.38	0.86 – 1.37	2.38 – 2.55

Table 2. The actual specific leaf weight based on SPAD range in selected fruit species

Range of SPAD readings	Actual specific leaf weight (g/m^2)					
	Banana	Carambola	Dokong	Durian	Mangosteen	Rambutan
≤ 20	28.3 – 31.1	31.4 – 40.4	61.3 – 75.4	63.8 – 67.6	–	62.2 – 70.3
21–35	29.6 – 43.2	34.4 – 48.7	80.0 – 107.4	57.9 – 82.9	59.4 – 122	61.8 – 81.1
36–55	41.5 – 55.8	45.4 – 58.6	87.3 – 108.9	70.6 – 118.1	99.0 – 134.2	80.6 – 109.6
≥ 55	56.2 – 61.6	57.9 – 68.6	99.6 – 112.9	100.5 – 109.9	119.8 – 203.5	100.7 – 132.9

SPAD reading and leaf chlorophyll concentration

Generally, there was an increasing trend of total chlorophyll concentration as SPAD values increased in all species (*Table 1* and *Figure 1*). Both the rate of change and concentration range (minimum and maximum) varied among species. Leaf chlorophyll concentrations ranged from 0.4–2.7, 0.7–2.5, 0.1–2.6, 0.1–2.4, 0.1–1.4 and 0.6–2.6 mg/g for banana, carambola, dokong, durian, mangosteen and rambutan respectively *(Table 1)*. The chlorophyll concentration for carambola and rambutan is consistently high, followed by banana, dokong and durian in the intermediate range and the lowest range is in mangosteen *(Table 1)*.

There was a significant ($p < 0.001$) positive relationship between SPAD readings with DMSO-extractable total leaf chlorophyll concentrations in all the six species studied *(Figure 1)*. Correlation coefficients for the linear regressions of these SPAD values against chlorophyll concentrations were 0.93, 0.93, 0.9, 0.93, 0.88, and 0.71 for banana, carambola, dokong, durian, mangosteen and rambutan respectively *(Figure 1)*. With the exception of rambutan ($r^2 = 0.71$), regression analyses revealed strong relationships (r^2 above 0.85) between total chlorophyll concentrations and SPAD readings in other crops, suggesting that the SPAD meter could be used to provide a rapid estimate of total chlorophyll concentration in the field.

SPAD reading and specific leaf weight

The range of observed SLW (g/m^2) for a given SPAD value was given in *Table 2*. Similar to chlorophyll concentration, there was also an increasing trend of SLW as SPAD values increased (*Table 2* and *Figure 2*). Specific leaf weight ranged from 28.3–61.6, 31.4–68.6, 61.3–112.9, 63.8–109.9, 59.4–203.5 and 62.2–132.9 g/m^2 in banana, carambola, dokong, durian, mangosteen and rambutan respectively *(Table 2)*. In general, banana and carambola had relatively lower SLW throughout based on observed ranges of SPAD values as compared to other fruit species.

Relationships between SPAD meter readings and specific leaf weight were linear with r^2 greater than 0.6 in all species. Correlation coefficients for the linear regressions of these SPAD values against SLW were 0.89, 0.84, 0.63, 0.79, 0.72, and 0.78 for banana, carambola, dokong, durian, mangosteen and rambutan respectively *(Figure 2)*.

Discussion

SPAD reading and leaf chlorophyll concentration

There was a highly significant, linear relationship between DMSO-extractable leaf chlorophyll and SPAD values of a portable chlorophyll meter (SPAD-502) shown in banana, carambola, dokong, durian, mangosteen and rambutan. The results on the linear relationship of SPAD reading and total chlorophyll concentration reported in this study are consistent with those reported in other tropical and subtropical crop species

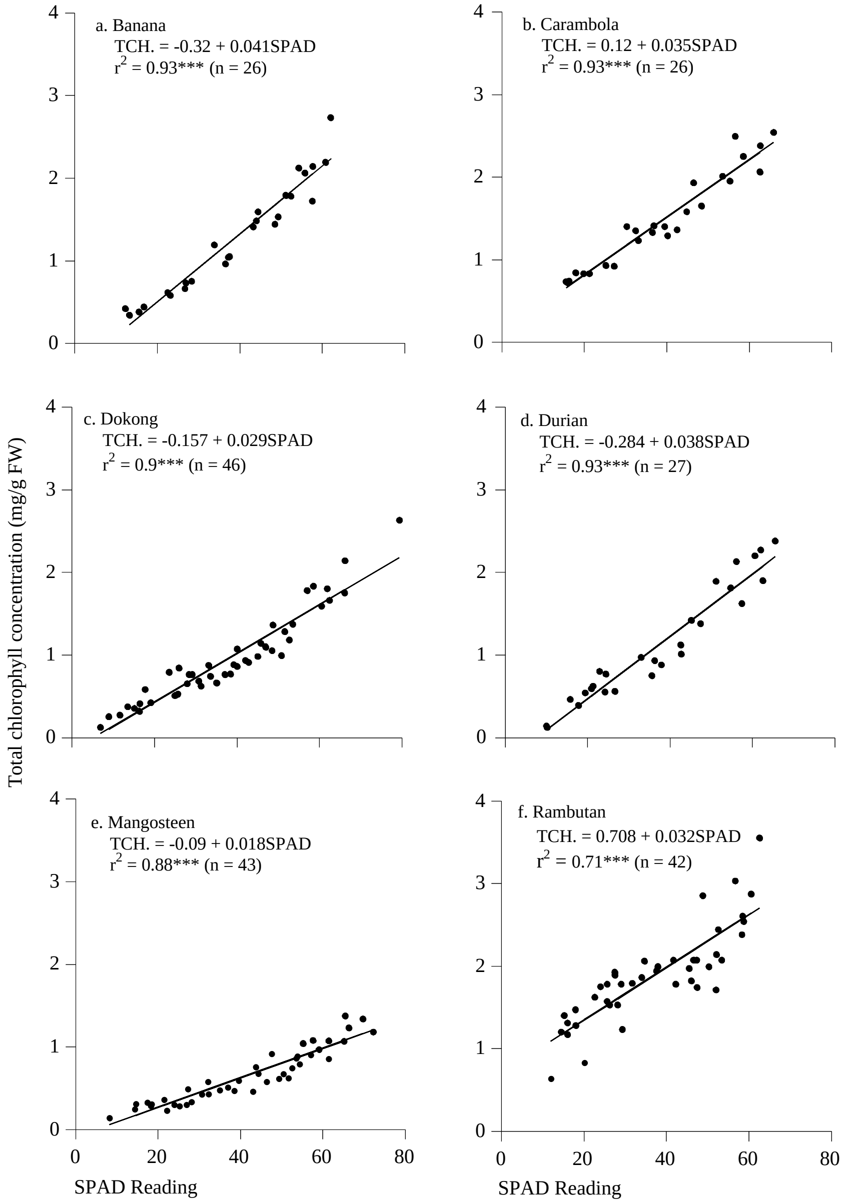

*Figure 1. Relationships between total chlorophyll concentration (mg chlorophyll/g tissue fresh weight) and SPAD readings for various fruit species (TCH. = Total chlorophyll concentration, ***p <0.001)*

(Schaper and Chacko 1991) as well as temperate species (Fanizza et al. 1991).

Chlorophyll a and b are important photosynthetic pigments. Absorption of radiation energy is a function of chlorophyll concentration; low chlorophyll concentration causes the photosynthetic rate to reduce. Leaf nitrogen is also found in chlorophyll molecules, lack of nitrogen also reduces the chlorophyll concentration. Environmental stress may increase carotenoid and reduces chlorophyll concentration (Hendry and Price 1993). These are basic primary processes that may influence chlorophyll concentration

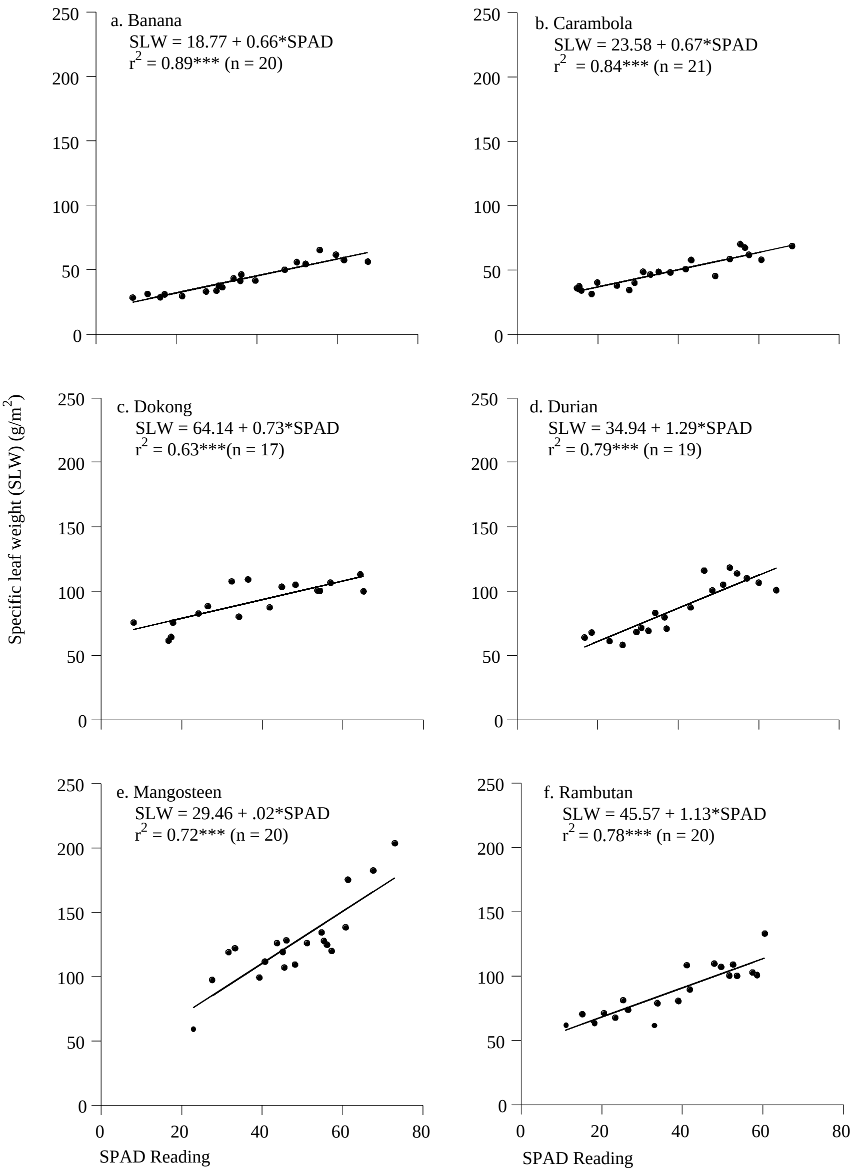

*Figure 2. Relationships between specific leaf weight (g/m²) and SPAD readings for various fruit species (SLW = Specific leaf weight, ***p <0.001)*

at the tissue level which, in turn, influence the whole plant and population performance. Some species or varieties are greener than others and will have higher readings. Stage of growth also affects the readings. Anything that causes the plant stress will affect the amount of chlorophyll in the plant and thereby affect the readings.

As indicated in this study, the relationship between the SPAD value and the measured chlorophyll varied from species to species. For any given range of SPAD values (≤ 20, 21–35, 36–55 and ≥ 55; *Table 1*), actual leaf chlorophyll concentrations varied with species. It is beyond the scope of this paper to systematically elucidate the cause for this variability. However, the result of this experiment corroborates findings of other workers on the variability of SPAD values with species (Fanizza et al. 1991), and leaf age (Wooge and Barden 1987).

Theoretically, leaf chlorophyll content is a direct indication of photosynthetic activity and carbohydrate production, and should therefore offer potential as a quick and easy method of growth measurement.

SPAD reading and specific leaf weight

The use of SPAD values to estimate SLW of banana, carambola, dokong, durian, mangosteen and rambutan was demonstrated in this study. Measurement with the Minolta 502 SPAD meter was rapid, simple and non-destructive. The positive relationships between SPAD and SLW were also demonstrated in rice (Peng et al. 1992), soybean (Thompson et al. 1996) and also barley (Araus et al. 1997).

Similarly with the SPAD-chlorophyll relationship, the relationship between SPAD value and SLW also varied from species to species. For any given range of SPAD values *(Table 2)*, actual SLW varied with species. Specific leaf weight is usually associated with morphological and physiological characteristics of the leaf. Apart from changes in chemical composition, the development during leaf ontogeny also involves leaf thickening and an increase in palisade depth (Ticha 1985; Ticha et al. 1985). In apple, it was reported that an increase in SLW during leaf ontogeny was attributed to structural changes (Wooge and Barden 1987).

Generally, SLW, nitrogen content per unit leaf area and also photosynthetic rate are interrelated. Specific leaf weight has been shown to be linearly correlated with nitrogen content per unit leaf area (DeJong and Doyle 1983), and with photosynthetic rate (DeJong 1985; Wooge and Barden 1987). The amount of leaf nitrogen per unit leaf area has also been shown to influence photosynthetic rates of many tree species (DeJong 1985; Syvertsen 1987). Reich et al. (1998) showed that maximum photosynthetic rate could be estimated based on the combination of SLW and leaf nitrogen. Changes in leaf anatomy and morphology during leaf ontogeny may substantially contribute to the respective changes in the course of leaf photosynthesis.

Regression models

As previously discussed, there are many factors affecting chlorophyll concentration and SLW. Different regression models are used for different species because some SPAD values may represent different SLW values and chlorophyll contents. Simple linear regression equations obtained in this study are sufficient to describe the relationships between SPAD and chlorophyll concentration and SLW. Other workers have shown other forms of relationship such as polynomial (Richardson et al. 2002; Torres Netto et al. 2002). The relationship between chlorophyll content and SPAD values obtained in this experiment could be further improved when chlorophyll was expressed on a leaf area rather than on a fresh weight basis (Marquard and Tipton 1987).

Leaf physiological indicator

In many ecophysiological studies, the needs to study environmental responses on leaf gas exchange or water relation under field condition are common. Choosing the right leaf samples to perform these measurements is critical. The description of 'fully expanded leaf' as frequently reported by many researchers is very subjective, mainly because it is merely based on colour, size or other visible description. On the other hand, the use of leaf samples with pre-determined physiological characteristic such as chlorophyll concentration or SLW will systematically minimise errors due to variability in physiological age, chemical composition or morphology. Handheld instrument such as this Minolta 502 SPAD meter can be easily used for quick and non-destructive determination of simple leaf physiological characteristic.

Within the same context, Earl and Tollenaar (1997) used SPAD reading to estimate leaf absorptance of the incident photon flux density in corn instead of time consuming technique using an integrating sphere and portable spectradiometer. In barley, Araus et al. (1997) estimate carbon isotope discrimination in field grown barley based on the positive correlation of SPAD reading with SLW and leaf nitrogen content. The use of SPAD values for indirect correlation with photochemical process in papaya leaves was also attempted (Torres Netto et al. 2002). In rice, it was suggested to use a constant SPAD value as threshold for timing of nitrogen fertilizer application for a given variety (Peng et al. 1995). However, the actual calibration is needed with respect to species, management practices as well environmental conditions (Richardson et al. 2002). The calibration is to establish the functional relationship between the two parameters or instruments, upon establishment, the time and space could be greatly improved. The users should bear in mind this is a tool to complement not to replace.

The capability of portable chlorophyll meter for rapid and non-destructive assessment of leaf chlorophyll concentration and SLW as demonstrated in this study can be made as a useful procedure for standardization of leaf samples for various ecophysiological measurements under field condition. Mathematical relationships of SPAD values with chlorophyll concentration and SLW should be expanded on other tropical plant species.

Conclusion

This study demonstrates the potential usage of the SPAD-502 chlorophyll meter for rapid field estimation of foliar total chlorophyll concentration and specific leaf weight for various tropical fruit species using mathematical equations. Leaf chlorophyll content and SLW can be used as plant growth indicators. These parameters can be correlated to many other important parameters such as leaf nitrogen content and photosynthetic rate. The good relationships between SPAD reading and chlorophyll concentration and between SPAD reading and SLW in the course of leaf growth would allow standardization of leaf samples for other physiological measurements based on direct SPAD meter readings. However for results to be accurate, SPAD values must be calibrated.

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Abstrak
Meter klorofil (SPAD 502) boleh digunakan untuk membuat anggaran kepekatan klorofil daun dan berat spesifik daun untuk beberapa spesies buah-buahan tropika. Objektif kajian ini adalah untuk menentukan kaitan bacaan SPAD dengan kepekatan klorofil daun dan berat spesifik daun semasa perubahan ontogeni daun. Korelasi yang signifikan (p <0.001) diperoleh untuk kedua-dua perhubungan nilai SPAD dengan kepekatan klorofil daun yang diekstrak dengan dimethylsulfoxide dan juga berat daun spesifik. Pekali korelasi untuk regresi linear antara bacaan SPAD dengan kepekatan klorofil ialah 0.93, 0.93, 0.9, 0.93, 0.88, dan 0.71 masing-masing untuk pisang, belimbing, dokong, durian, manggis dan rambutan. Manakala pekali korelasi untuk regressi linear antara bacaan SPAD dengan berat spesifik daun pula ialah 0.89, 0.84, 0.63, 0.79, 0.72, dan 0.78 masing-masing untuk pisang, belimbing, dokong, durian, manggis dan rambutan. Persamaan regresi untuk membuat anggaran klorofil dan berat spesifik daun dengan kaedah tanpa pemusnahan tisu dibentuk untuk setiap spesies. Kegunaan meter klorofil sebagai petunjuk fisiologi daun juga dibincangkan.

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