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RESEARCH ARTICLE

Rapid and nondestructive estimations of chlorophyll concentration in date palm (*Phoenix dactylifera* L.) leaflets using SPAD-502+ and CCM-200 portable chlorophyll meters

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is the oldest known tree grown in the world's arid regions. It has been cultivated for centuries because of its enormous cultural, agricultural, and environmental benefits. Recently date palm groves have been declined drastically due to anthropogenic, abiotic and, biotic stresses. Attaining sustainable farming and optimum crop production requires frequent monitoring of the physiological status of date palms. This demands rapid and non-destructive estimation of chlorophyll concentration in date palm leaflets overtime. In this study, four date palm cultivars, exhibiting distinct concentrations of chlorophyll, were used to assess the potential of using SPAD-502+ and CCM-200 portable chlorophyll meters to estimate the concentration of chlorophyll in date palm leaflets. Regression analyses were performed to model the relationship between the absolute concentration of chlorophyll measured *in vitro* and the optic indices of the two portable chlorophyll meters (SPAD and CCI). The results revealed that polynomial and power prediction models, which demonstrated remarkably close fits to each another, are the best functions to parameterize the relationships. The calibration models developed in this study were very strong and recorded high coefficient of determinations along with low relative errors for both the cultivar-specific fits ($R^2 \geq 0.89$; Error % ≤ 18.3) as well as the generic species-specific fits ($R^2 \geq 0.822$; Error % ≤ 25.0). The results confirmed that both SPAD-502+ and CCM-200 are equally effective tools for rapid and nondestructive estimation of chlorophyll concentration in date palm leaflets.

Keywords: CCM-200; chlorophyll; date palm; leaflets; SPAD-502+

INTRODUCTION

Chlorophyll is a pivotal photosynthetic pigment, which is involved in light harvesting and transforming during light-dependent phase of photosynthesis (Steele et al., 2008; Pavlovic et al., 2014). Plant's ability to absorb and trap light energy during the onset of photosynthesis is proportional to the concentration and activity of chloroplast pigments, especially chlorophyll (Pal et al., 2012). This indicates that photosynthetic capacity and hence plants growth and productivity are dependent on the concentration of chlorophyll in plant leaves. Accordingly, measuring chlorophyll concentration was the emphasis of researchers exploring plants physiological status under normal and stressed growing conditions (e.g. Taïbi et al., 2016; Bresson et al., 2017; Melo et al., 2017; Mattila et al., 2018; Sharma et al., 2019; Shareef et al., 2020; Bresson et al., 2017).

Maintaining optimum plant growth and development under various growing conditions requires frequent assessment of chlorophyll concentration, which reflects plant physiological status and health. This demands rapid, easy, and nondestructive techniques that enable repeated estimation of chlorophyll concentration of the same samples overtime (Pal et al., 2012). Traditional techniques used to quantify chlorophyll concentrations are expensive, tedious, and time-consuming. They depend on destructive intracellular pigment extraction, which demands cell disruption and rely on various organic solvents, many of which are toxic and expensive (Minocha et al., 2009; Putra et al., 2017). Accordingly, optical devices have been developed as easy,

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nondestructive and timesaving alternatives to the traditional extraction methods currently in use (Markwell et al., 1995; Sibley et al., 1996; Silla et al., 2010).

Among the available optical devices, SPAD-502+ (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan) and CCM-200 (Opti-Sciences, Inc., Hudson, NH, USA) are widely used to estimate foliage chlorophyll content. These portable chlorophyll meters (PCMs) have been used to estimate the chlorophyll content of a variety of crop plants; including, maize (*Zea mays* L.), tomato (Solanum lycopersicum L.), soybean (*Glycine max* L. Merr.), spring wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.) and cucumber (*Cucumis sativus* L.) (Kalaji et al., 2016; Dong et al., 2019; de Souza et al., 2020). They have been used to estimate chlorophyll content in hardwood plant species such as sugar maple (*Acer saccharum* Marsh.) and palm trees such as oil palm (*Elaies guineensis*) (Berg and Perkins, 2004; Sim et al., 2015).

Both SPAD-502+ and CCM-200 chlorophyll meters are designed to provide optic indices (SPAD and CCI, respectively) by measuring red (650–660 nm) and near-infrared (930–940 nm) light transmittance through plant leaves (Richardson et al., 2002). The red light, which is considered as an index band, corresponds to chlorophyll light absorption zone and hence correlates with the concentration of chlorophyll in the measured area. In contrary, the near-infrared light, which is considered as a reference band, is used as a reference value to considers the variation in leaf structure (Silla et al., 2010; Dong et al., 2019).

The derived SPAD and CCI optic indices are relative values that corresponds to the actual concentration of chlorophyll in plant leaves. Therefore, estimating the actual concentration of chlorophyll in absolute units of concentration demands modeling the relationship between the optic indices and the absolute chlorophyll concentration measured *in vitro* and construct the best calibration fit (Parry et al., 2014). Previous research indicated that the mathematical models constructed to illustrate the relationship between SPAD-502+ and CCM-200 readings and actual foliage chlorophyll content varies across plant species, growing conditions, and cultivars within the same species (Hawkins et al., 2009; Parry et al., 2014). Accordingly, various prediction equations have been developed to quantify chlorophyll concentration for individual species using SPAD-502+ and CCM-200 chlorophyll meters. Several attempts revealed that the beforementioned relationships are parametrized as linear relationships (Piekielek, et al., 1995; Peng et al., 1996; Cassol et al., 2008); whereas other studies indicated nonlinear relationships (Uddling et al., 2007; Viera Silva et al., 2016; Padilla et al., 2018).

Date palm (*Phoenix dactylifera* L.) is the oldest known tree grown in the world's arid regions (Niazi et al., 2017). It has been cultivated for centuries because of its enormous cultural, agricultural, and environmental benefits (El-Juhany, 2010). Dates, the fruit of the tree, possess high socioeconomic, nutritional and medicinal values in the main date producer countries, particularly in North Africa and the Arabian Gulf region (El-Juhany, 2010; Erskine et al., 2014; Taha and Al Ghtani, 2015; Almansoori et al., 2015). Unfortunately, date palm groves have been declining drastically due to anthropogenic, abiotic and biotic stresses. It has been documented that date palm stress tolerance is greater than many other crop plant species. Nevertheless, elevated temperature, soil salinity, prolonged drought seasons, pest infestations, and diseases challenge their growth and productivity. Such stresses adversely affected the growth of date palm trees and caused considerable quality and quantity yield loss (Almansoori et al., 2015; Yaish and Kumar, 2015; Al-Dosary et al., 2016).

Earlier studies reported significant decline in the concentration of chlorophyll upon date palm exposure to abiotic and biotic stresses (Shareef, et al., 2020; Abbas et al., 2014). Therefore, frequent assessment of chlorophyll content of the active photosynthetic leaflets overtime is a fundamental practice to sustain optimum growth and productivity levels. This demand easy, rapid and nondestructive techniques to evaluate the plant's physiological status repeatedly. However, date palms are characterized by having stiff photosynthetic leaflets with thick adaxial cuticle. The stiffness of the leaflets result from the high concentrations of callouses and lignin and the richness in microfibers (Rivera et al., 2008; Su et al., 2010). The stiffness of the leaflets hinders measuring chlorophyll concentration using traditional chemical extraction methods (Wu et al., 2002). It is indubitable that the traditional techniques do not fulfill the requirements for frequent monitoring of date palm physiological status, which aims for achieving sustainable crop production. This necessitates frequent and repeated assessment of chlorophyll contents of the same sample overtime.

Therefore, PCMs such as SPAD-502+ and CCM-200, can be the option for repeated estimation of the concentration of chlorophyll in date palm leaflets to evaluate the physiological status under various growing conditions. Research exploring the potential of exploiting PCMs to estimate the concentration of chlorophyll in date palm leaflets is very limited. The only research in this field conducted by Esechie and Al-Falahi (2007) revealed a strong positive and significant correlation between SPAD optic indices and acetone extracted chlorophyll (R^2 value ranged from 0.86 to 0.93). However, the relationship between SPAD-502+ and CCM-200 and absolute chlorophyll

concentrations have not been investigated and calibration curves that tend to quantify chlorophyll concentration in date palm leaflets have not been established.

Accordingly, the current study was conducted to address the following aims: (1) the potential of using SPAD-502+ and CCM-200 portable chlorophyll meters to estimate the concentration of chlorophyll in date palm leaflets, (2) constructing best calibration fits for the studied date palm cultivars, (3) the potential of using the pooled data to generate generic species-specific calibration fits for date palm as an independent plant species, and (4) developing a generic conversion equation for the optic indices derived from the two PCMs used in this investigation (SPAD and CCI).

MATERIALS AND METHODS

Sampling plant materials

Fronds of various developmental stages were collected from four date palm cultivars (Khalas, Khunaizi, Barhi, and Hilali) coexisted in a managed private farm located in Al-Jasra (26.1616° N, 50.4567° E) in the Northern Governorate, the Kingdom of Bahrain. For each cultivar, five of 7-10 years old healthy date palm trees were selected as the sources of the fronds. Sampling was accomplished acropetally from the crown base to the apex of each palm tree. Fronds ranged from senescing, pre-senescing, fully expanded to recently mature were collected. Non-photosynthetically active fronds including the immature heterotrophic and very dry senescent fronds were excluded from the current study. To prevent fluctuation in leaflets water content and circumvent the effect of light intensity on chloroplast movement (Parry *et al*., 2014) samples were collected in the early mornings. Sampling was exploited in December 2018 with an average daily temperature of 21.6ºC and relative humidity of 67% (World Meteorological Organization, 2018). Samples were immediately wrapped in plastic bags and kept refrigerated prior to analysis.

Individual leaflets were separated from the rachis avoiding the basal and distal leaflets of each frond. The leaflets of the five trees of each cultivar were bulked, washed thoroughly under running tap water, followed by a final rinse with distilled water and blot dried. After that, thirty leaflets were selected visually according to the gradient in their color from the most healthy and green leaflets obtained from fully expanded and recently mature fronds to the most chlorotic and necrotic leaflets obtained from the naturally senescing fronds. Each leaflet was divided longitudinally into two halves throughout the midrib. Readings were performed on both sides of the leaflet and then averaged to obtain one reading per leaflet.

Measuring SPAD and CCI optic indices

SPAD and CCI optic indices were measured using two portable handheld chlorophyll meters, SPAD-502+ (Minolta Camera Co., Osaka, Japan) and CCM-200 (CCM-200 plus model, Opti-Sciences, Inc., Hudson, NH, USA). To accommodate for CCM-200 sensing aperture (71mm^2) measurements were performed along the widest midsection of the leaflets avoiding the narrow distal and proximal ends. On each side of the leaflet, two spots were marked, and five readings were recorded on each spot, using SPAD-502+ and CCM-200 separately. Readings then were averaged and used as a representative single SPAD and CCI values of each leaflet.

Measuring the absolute concentration of chlorophyll

Immediately after recording the SPAD and CCI optic indices, samples were excised accurately from the same spots where readings were performed. Sample disks from each side of the leaflet were bulked separately and fresh weight was recorded prior to storing at –19ºC for a maximum of five days. To measure the absolute concentrations of chlorophyll, the sample disks were first immersed in 1ml chilled absolute acetone supplemented with 50mg $CaCO_3$. Then, samples were sonicated in ice for 5 minutes at medium intensity (160 W) using standard sonication system (Diagenode Bioruptor Standard Sonication System Control Unit UCD-200TM-EX 115VAC, Japan) before being vigorously ground into a dry powder with 50mg acid washed sand. Subsequently, chlorophyll pigments were extracted using 80% acetone and centrifuged at 5000 rpm for 20 min at 4ºC. Then, the absorbance of the extract was measured at 663.2 and 646.8nm using UV-spectrophotometer (GENESYS™ 20 UV-spectrophotometer, SHIMADZU). Leaflets total chlorophyll concentration (Chl) was calculated as the sum of the concentrations of chlorophyll a (Chl_a) and chlorophyll b (Chl_b) determined using the following equations (Lichtenthaler and Buschmann, 2001):

$$Chl_a = (12.25A_{663.2} – 2.79A_{646.8}) * V/(LA \text{ or } FW)$$
$$Chl_b = (21.50A_{646.8} – 5.10A_{663.2}) * V/(LA \text{ or } FW)$$

where A is the absorption at the referenced wavelength (nm); V is the volume of the solvent (mL); LA is the total leaflets area used to calculate Chl per unit area (μg cm^{-2}) and denoted subsequently in text by (Chl_{area}); FW is the total leaflets fresh weight used to calculate Chl per unit fresh weight (μg g_{fw}^{-1}) and denoted subsequently by (Chl_{fw}). Measurements were repeated on both sides of the leaflets and averaged to obtain a representative value per leaflet.

Statistical analysis

To examine the differences among the studied cultivars in chlorophyll contents at the onset of the investigation, descriptive statistics including: Arithmetic mean, standard

deviation, minimum, maximum and coefficient of variation (CV) were calculated for the concentration of total chlorophyll per unit area (Chl_{area}), the SPAD and the CCI readings for each individual cultivar and for all cultivars combined. The differences in Chl_{area} across the cultivars were analyzed using ANOVA. Regression analysis were performed to obtain the best mathematical models relating Chl_{area} in date palm leaflets to the SPAD and CCI readings. In this study both the fit based on a polynomial function (linear or quadratic) and the fit based on a power function were analyzed. The power fit was obtained by applying a log-log transformation, which linearized the relationship between the PCM readings and the measured leaflets Chl_{area}. The accuracy of the fitted models was assessed using the coefficient of multiple determination (R^2), the root of the mean square error (RMSE), the bias (Bias), the standard error of prediction corrected for the bias (SEPC), and by the relative error (Error %) defined as the ratio of the SEPC relative to the mean of Chl_{area} (Cerovic et al., 2012).

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\hat{y}_i - y_i\right)^2}$$

$$Bias = \frac{1}{n}\sum_{i=1}^{n}\left(\hat{y}_i - y_i\right)$$

$$SEPC = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\hat{y}_i - y_i - Bias\right)^2}$$

RESULTS

Variability of chlorophyll content among the studied cultivars

The results of the descriptive statistics of the absolute concentrations of total chlorophyll obtained via *in vitro* acetone extraction method are depicted in Table 1. These results indicate that the leaflets of the four investigated date palm cultivars possessed different Chl, when expressed both as Chl_{area} or Chl_{fw}. This was supported by ANOVA analysis, which showed significant differences among the means of the Chl_{area} ($F(3,114) = 8.244$, $P < 0.0001$) and among the means of the Chl_{fw} ($F(3,114) = 9.00$, $P < 0.0001$) across the studied cultivars. Fisher's protected LSD multiple comparisons showed similar results for the mean differences when Chl_{area} and Chl_{fw} were considered. In both cases, the Khalas cultivar, which had the highest means of (Chl_{area}: 57.08 μg/cm^2; Chl_{fw}: 1129.25 μg/g) differed significantly (P<0.05) from Khunaizi, Barhi and Hilali cultivars. Further, the Khunaizi cultivar, which had the least means of (Chl_{area}: 31.25 μg/cm^2; Chl_{fw}: 616.16 μg/g) differed significantly (P<0.05) from both the Barhi and the Hilali. Contrariwise, the means of the Chl of Barhi (Chl_{area} 41.55 μg/cm^2; Chl_{fw}: 837.9 μg/g) and Hilali (Chl_{area} 42.22 μg/cm^2; Chl_{fw}: 835.82 μg/g) were almost identical to each another with no significant statistical differences (Chl_{area}: P = 0.898; Chl_{fw}: P = 0.983).

Table 1: Summary of the descriptive statistics of measured leaflets chlorophyll contents of four date palm cultivars (Khalas, Khunaizi, Barhi, Hilali) and pooled results using PCM and *in vitro* acetone extraction methods

	Mean ± SD	CV (%)	Minimum	Maximum
Cultivar				
Khalas				
SPAD	56.86 ± 17.28	30.39	14.10	74.80
CCI	45.05 ± 24.94	55.36	4.20	83.74
Chl_{area} (μg cm^{-2})	57.08 ± 24.36	42.68	6.46	86.59
Chl_{fw} (μg gfw^{-1})	1129.25 ± 449.10	39.77	157.57	1602.67
Khunaizi				
SPAD	48.51 ± 16.89	34.82	17.50	77.60
CCI	40.16 ± 25.07	62.43	4.70	82.22
Chl_{area} (μg cm^{-2})	31.25 ± 16.22	51.90	5.52	59.40
Chl_{fw} (μg gfw^{-1})	616.16 ± 300.38	48.75	124.29	1086.16
Barhi				
SPAD	53.11 ± 17.33	32.63	14.70	77.00
CCI	43.62 ± 25.79	59.12	4.32	82.18
Chl_{area} (μg cm^{-2})	41.55 ± 20.20	48.62	8.11	85.29
Chl_{fw} (μg gfw^{-1})	837.90 ± 398.36	47.54	172.01	1671.84
Helali				
SPAD	57.48 ± 17.01	29.59	20.90	78.30
CCI	48.22 ± 25.18	52.22	5.82	83.22
Chl_{area} (μg cm^{-2})	42.22 ± 18.75	44.41	8.85	69.69
Chl_{fw} (μg gfw^{-1})	835.82 ± 360.99	43.19	171.54	1403.34
Pooled				
SPAD	53.94 ± 17.29	32.05	14.10	78.30
CCI	44.22 ± 25.10	56.76	4.20	83.74
Chl_{area} (μg cm^{-2})	42.91 ± 21.86	50.94	5.52	86.59
Chl_{fw} (μg gfw^{-1})	852.62 ± 417.87	49.01	124.29	1671.84

CV is the coefficient of variation, measured as the ratio of the standard deviation to the mean

Khalas cultivar exhibited the smallest variation (CV: Chl_{area} 42.68; Chl_{fw} 39.77) followed by Hilali (CV: Chl_{area} 44.42; Chl_{fw} 43.19), Barhi (CV: Chl_{area} 48.62; Chl_{fw} 47.54) and the highest variation (CV: Chl_{area} 51.91; Chl_{fw} 48.75) was evident in Khunaizi (Table 1). The pooled Chl of the four cultivars, which exhibited means of (Chl_{area} 42.91 μg/cm^2; Chl_{fw} 852.62 μg/g) and ranges of (Chl_{area} 5.52- 86.59 μg/cm^2; Chl_{fw} 124.29- 1671.84 μg/g), showed CV values of (CV: Chl_{area} 50.94; Chl_{fw} 49.01), which is comparable to the variability of Khunaizi and Barhi. For each of the four

cultivars, along with the pooled results, the CV of Chl_{area} and Chl_{fw} were remarkably close to each another (Table 1).

On the other hand, the statistical analysis of the SPAD and CCI relative optic indices, revealed significant variations between the readings of the two PCMs used in the current investigation (Table 1). The readings of SPAD-502+ exhibited narrower range (Range: 14.1-78.3) and smaller variation (CV:32.05) compared to that of CCM-200 meter (Range: 4.2-83.74; CV: 56.75). Similar to the pooled results, the SPAD-502+ readings for each specific cultivar exhibited less variation compared to that of CCM-200 readings.

The relationship between in vitro measured chlorophyll concentration and PCMs optic indices

Strong relationships ($P < 0.0001$) between Chl_{fw} and Chl_{area} and the readings of the two PCMs, SPAD-502+ and CCM-200, were evident across all of the investigated cultivars and the pooled data. The models constructed to illustrate the relationships between Chl_{area} and PCMs optic indices exhibited almost similar proportion of variance (R^2) and relative error (Error %) as Chl_{fw} across all of the cultivars and the pooled data (R^2 difference (-0.015) - 0.036%; relative error difference range: (-1.6) - 0.4%). In addition, strong linear relationship was confirmed between Chl_{area} and Chl_{fw} pooled data (R^2 0.978; relative error 7.49%).

$$Chl_{area} = -1.162 + 0.052\ Chl_{fw}$$

This indicates that the two units used to measure Chl are compatible and interchangeable. Accordingly, only the results of the regression analysis obtained for Chl_{area} are presented in this study.

Quadratic and linear (polynomial) as well as power functions were the superior prediction models that best illustrated the relationships between the *in vitro* measured Chl_{area} and the PCMs readings (Table 2, Fig 1). The explained proportion of variance (R^2) and the relative error percentage (Error %) indicated that the efficiency of the developed prediction models used to estimate Chl_{area} in the leaflets of date palm cultivars were very strong for both PCMs (R^2 range: 0.893-0.965; relative error range: 11.2%-18.1%). The two prediction models provided strong and, in some cases, identical fits to the data obtained for each cultivar (Table 2, Fig1). The only exception was noted when the Khalas cultivar was considered. The quadratic models exhibited stronger fits when chlorophyll content was estimated using CCM-200 compared to the power model (Chl_{area}: power model R^2 0.930, relative error 17.1% versus quadratic model R^2 0.960, relative error 8.4%).

Variations between the cultivars were also evident when the polynomial relationships were investigated. The *in vitro* measured Chl_{area} for Khalas and Khunaizi showed strong linear fits with SPAD-502+ readings, whereas Barhi and Hilali exhibited quadratic fits. In contrary, CCM-200 readings exhibited quadratic relationships with Chl_{area} for all of the cultivars, except Hilali, which showed linear relationship with Chl_{area} (Table 2, Fig 1).

Table 2: Regression analysis between readings from portable chlorophyll meters (SPAD and CCI) and *in vitro* measured leaflets total chlorophyll concentration in (µg cm^{-2}) denoted by Chl_{area} using logarithmic transformation power model and linear or quadratic polynomial models.

Cultivar	Models	R^2	RMSE	Bias	SEPC	Error (%)
Khalas	$Chl_{area} = 0.084\ SPAD^{1.601}$	0.951	6.394	0.454	6.378	11.2
	$Chl_{area} = -20.201 + 1.359\ SPAD$	0.930	6.351	0.001	6.351	11.1
	$Chl_{area} = 2.448\ CCI^{0.835}$	0.930	9.859	- 1.312	9.772	17.1
	$Chl_{area} = -0.015\ CCI^2 + 2.261\ CCI - 5.300$	0.960	4.786	- 0.033	4.786	8.4
Khunaizi	$Chl_{area} = 0.070\ SPAD^{1.551}$	0.895	5.683	0.692	5.641	18.1
	$Chl_{area} = -13.182 + 0.900\ SPAD$	0.904	5.738	0.561	5.710	18.3
	$Chl_{area} = 1.605\ CCI^{0.808}$	0.959	4.061	0.139	4.059	13.0
	$Chl_{area} = -0.004\ CCI^2 + 0.967\ CCI + 1.007$	0.944	3.816	- 0.072	3.815	12.2
Barhi	$Chl_{area} = 0.165\ SPAD^{1.380}$	0.910	7.039	0.765	6.997	16.8
	$Chl_{area} = 0.011\ SPAD^2 + 0.004\ SPAD + 7.066$	0.894	6.476	0.044	6.476	15.6
	$Chl_{area} = 3.243\ CCI^{0.685}$	0.965	5.713	0.396	5.699	13.7
	$Chl_{area} = -0.002\ CCI^2 + 0.859\ CCI + 8.1837$	0.958	5.890	0.988	5.807	14.0
Hilali	$Chl_{area} = 0.108\ SPAD^{1.461}$	0.893	6.880	1.573	6.697	15.9
	$Chl_{area} = 0.014\ SPAD^2 - 0.396\ SPAD + 14.238$	0.949	6.427	1.534	6.356	15.1
	$Chl_{area} = 2.478\ CCI^{0.737}$	0.938	5.807	1.203	5.681	13.5
	$Chl_{area} = 7.389 + 0.724\ CCI$	0.953	5.991	0.755	5.834	13.8
Pooled	$Chl_{area} = 0.087\ SPAD^{1.538}$	0.874	9.789	1.035	9.734	22.7
	$Chl_{area} = 0.010\ SPAD^2 + 0.162\ SPAD + 2.551$	0.822	9.773	0.721	9.745	22.7
	$Chl_{area} = 2.287\ CCI^{0.776}$	0.867	10.778	1.040	10.728	25.0
	$Chl_{area} = -0.004\ CCI^2 + 1.123\ CCI + 2.701$	0.826	10.509	0.231	10.507	24.5

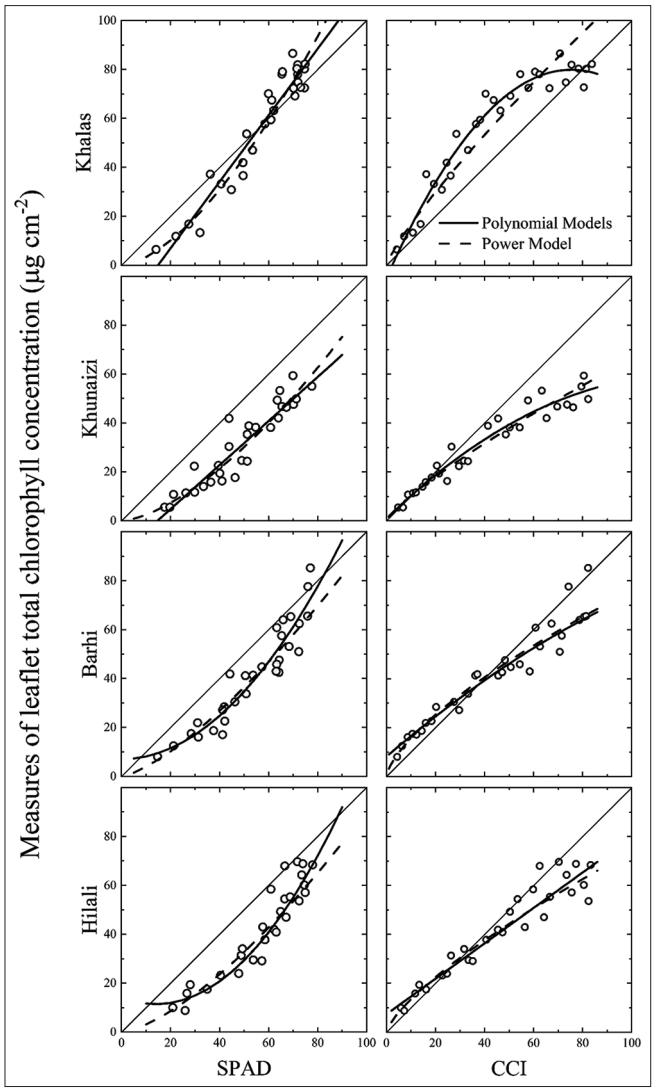

Fig 1. The relationships between portable chlorophyll meter readings (SPAD, CCI) and *in vitro* measured total chlorophyll concentrations (Chl_{area}) in four date palm cultivars.

The data depicted in Fig 2 indicate that the models developed for the Khalas exhibited the highest slopes. Accordingly, for a given reading of the PCMs > 20, the power models developed for Khalas cultivar yielded the highest Chl_{area}, whereas the Khunaizi models gave the least Chl_{area}. Both Barhi and Hilali prediction models read relatively similar Chl_{area} in between the ranges of the Khalas and Khunaizi cultivars (Fig 2). Similar results were obtained using the polynomial models with respect to the CCI reading. However, they were recognized for the readings of SPAD > 60 in relation to Chl_{area}.

The polynomial models for the Khalas cultivar, which exhibited the best fit by expressing higher R^2 and lower Error %, cannot be used to estimate Chl_{area} above 80 µg cm^{-2} using CCM-200. Readings exceeding 60-70 CCI units correlated with lower concentrations of Chl_{area}. This indicates

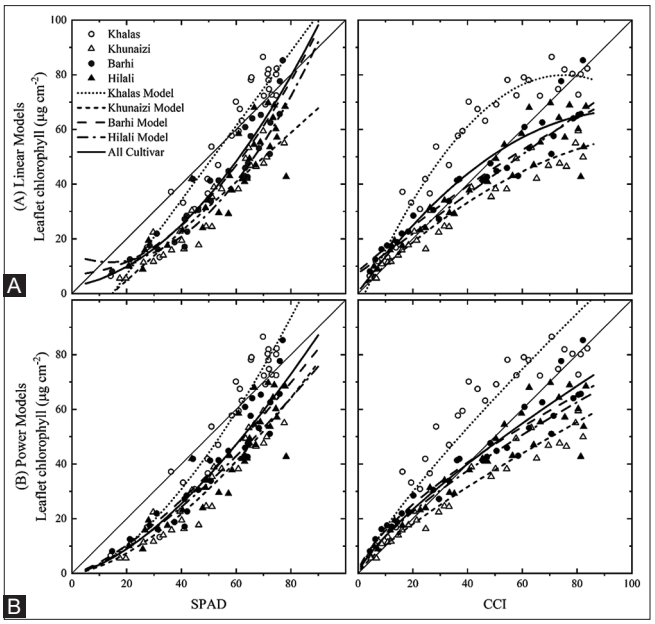

Fig 2. The relationships between portable chlorophyll meter readings (SPAD, CCI) and *in vitro* measured chlorophyll concentrations (Chl_{area}) of pooled data of four date palm cultivars using: (A) linear or quadratic polynomial models, (B) power models.

the interference of other factor on the CCM-200 light absorption beside the chlorophyll contents (Fig1, Fig 2A).

Although the regression models (Table 2, Fig 1) suggested the validity of exploiting different prediction models to estimate the Chl_{area} for the diverse date palm cultivars, the models developed for the pooled data of the four cultivars revealed strong relationships as well ($P<0.0001$). As with individual cultivars, the relationships of the pooled data were best described by quadratic and power relationships (Table 2). The pooled data revealed slightly higher R^2 for the power models compared to the polynomial models; yet both exhibited almost equal Error %. This suggests that the power models were slightly stronger than the quadratic models for the pooled data. As illustrated in Fig 2, the pooled data of the four cultivars were more clustered at lower SPAD and CCI values and diverged as they increased. For both PCMs, the data dispersion was more evident in the polynomial models compared to the power models. The polynomial models conveyed interactions between the slopes of the cultivars even at low values of SPAD and CCI (Fig 2A).

Relationship between SPAD-502+ and CCM-200 meters

Combined readings were used to model the relationship between SPAD and CCI optic indices. Strong curvilinear relationships were verified between the readings of the two PCMs (Table 3). Power and the second order polynomial models best explained the relationships between the readings of the two devices (Table 3, Fig 3). The R^2 values

Table 3: Regression analysis between readings from SPAD-502+ and CCM-200 meters using logarithmic transformation power model and quadratic model.

Model	Converting SPAD to CCI units			Converting CCI to SPAD units		
	Equation	R^2	Error (%)	Equation	R^2	Error (%)
Power	$CCI = 0.017\ SPAD^{1.941}$	0.949	15.30	$SPAD = 8.926\ CCI^{0.489}$	0.949	7.57
Quadratic	$CCI = 0.18\ SPAD^2 - 0.370\ SPAD + 6.242$	0.943	14.14	$SPAD = -0.007\ CCI^2 + 1.269\ CCI + 16.219$	0.946	7.45

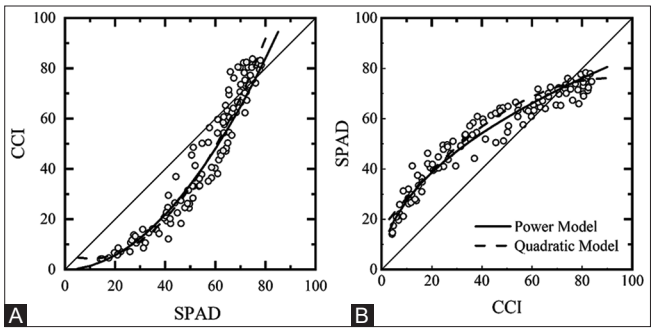

Fig 3. Plot of the regression relationship of: (A) CCI on SPAD, and (B) SPAD on CCI.

supported by Error % indicate the high efficiency of the developed models to convert SPAD to CCI and vice versa when used to estimate the Chl_{area} of date palm leaflets ($R^2 > 0.940$; relative error < 15.30%).

DISCUSSION

Attaining sustainable farming and optimum crop production requires monitoring the physiological status of date palm trees over time. The concentration of chlorophyll, which is the key photosynthetic pigment, has been frequently used to evaluate plants health under various environmental conditions (Gitelson et al., 2003; Pavlovic et al., 2014; Li et al., 2018; Agathokleous et al., 2020). In this study, four date palm cultivars, exhibiting distinct chlorophyll concentrations, were used to assess the potential of using SPAD-502+ and CCM-200 portable chlorophyll meters for rapid and non-destructive prediction of chlorophyll concentration in date palm leaflets, also known as pinnae.

Plynomial (linear and quadratic) and power prediction models, which demonstrated remarkably close fits to each another especially at the intermediate ranges, are the best fits for calibrating the relationships between the *in vitro* measured Chl_{area} and SPAD and CCM relative optic indices. Both functions exhibited similar coefficient of determinations (R^2) and relative errors (Error %), indicating that the two models are equally accurate in predicting Chl_{area} in date palm leaflets. The only exception was recorded for Khalas cultivar in which the quadratic model represented a stronger calibration fit for CCM-200 chlorophyll meter compared to the power function. In literatures, similar relationships were frequently parameterized by polynomial, power, exponential and homographic models (Uddling et al., 2007; Coste et. al., 2010; Casa et al., 2014; Viera Silva et al., 2016; Padilla et al., 2018). However, the exponential model was not considered in this study because it did not explain the convexity in the relationship between the CCM-200 readings and Chl_{area}; whereas the homographic model was not indorsed because it forces the curve to pass through the origin, which contradicts the actual *in vitro* measured Chl_{area}.

Aligned with previous findings (Uddling et al., 2007; Cerovic et al., 2012; Viera Silva et al., 2016; Padilla et al., 2018), the current investigation revealed that the relationships between Chl_{area} and PCMs optic indices (SPAD and CCI) have more tendency towards non-linear than linear relationships. However, the quadratic term was not significant when modelling the relationship of the total chlorophyll content to the SPAD-502+ readings in case of the Khalas and Khunaizi cultivars ($P = 0.375$ and 0.185 respectively) and to the CCM-200 readings in the case of the Hilali cultivar ($P = 0.160$), the latest relationships were best described by linear functions. Although the abovementioned linear functions exhibited similar fit with the data as the correspondence power models and they are in accordance with the finding of previous investigations (Piekielek, et al., 1995; Peng et al., 1996; Cassol et al., 2008), their accuracy in predicting the Chl_{area} in date palm leaflets need to be treated with cautious, especially at the two extremes of the functions. The results show the tendency of the linear models to under predict Chl_{area} at the lower ranges of the measured optic indices and over predicts Chl_{area} at the higher ranges. In addition, when PCMs indices correspond to zero values, the prediction linear models recorded negative Chl_{area} values, which did not reflect the *in vitro* measured Chl_{area}. It was evident that both SPAD-502+ and CCM-200 meters were unable to measure the low concentrations of chlorophyll in the senescent date palm leaflets due to the extensive amount of dense cell wall that interferes with the absorption and transmission of the incident light. In accordance with the current findings, Uddling et al. (2007) favored the nonlinear exponential models over the linear models to avoid under prediction of chlorophyll concentration and acquiring negative values at the lower ranges of SPAD.

It has been documented that the relationship between chlorophyll concentration and the optic indices of the PCMs depends on the homogeneity of chlorophyll distribution within the measured area (Uddling et al., 2007; Novichonok et al., 2016). Homogeneous distribution of chlorophyll in plant leaves is projected by linear relationships, whereas non-homogeneous distribution of chlorophyll is anticipated by nonlinear functions. The homogeneity of chlorophyll distribution though depends on leaflets internal architecture. It depends on thylakoid arrangement in chloroplast, distribution and orientation of chloroplasts within individual cells and cells arrangement in the leaf (Fukshansky et al., 1993). In Addition, Uddling et al 2007 deemed that the relatively translucent leaf veins are important cause of non-uniform distribution of chlorophyll in plant's leaf. Being a monocotyledonous plant, the blades of date palm leaflets are characterized by parallel veins. It was evident that their frequency, distribution, shape, and size vary among the different cultivars (Arinkin et al., 2014). The variation in the blade's internal architecture affects the proportion of light absorption by the leaflets and hence impacts the values of the measured optic indices derived by the two PCMs used in this study.

As expected, stronger relationships were evident for individual cultivar-specific fits ($R^2 \geq 0.89$; Error % ≤ 18.3) compared to the generic species-specific fits ($R^2 \geq 0.822$; Error % ≤ 25.0). Moreover, significant variations were evident between the slopes of the cultivar-specific fits derived for the four studied cultivars. Khalas cultivar, which recorded the highest mean of Chl_{area}, exhibited relationships that acquired the highest slopes; whereas Khunaizi cultivar, which recorded the least mean of Chl_{area}, exhibited relationships that acquired the lowest slopes. This indicates that the relationships between Chl_{area} and the PCMs optic indices is cultivar specific. This finding is aligned with prior investigations reported distinct relationships for the cultivars of the same species (Jifon et al., 2005; Parry et al., 2014). The differences denoted between the slopes of the calibration fits derived for the studied cultivars points out the sieve effect resulted from the variation in Chl_{area} between the studied cultivars.

It has been reported that the increase in the concentration of chlorophyll in plant cells is not always associated with increase in number of chloroplasts. Instead, chlorophyll concentration builds within the thylakoid membranes of the existing chloroplasts causing sieve effect (Slattery et al., 2016). Sequestering excess chlorophyll in the chloroplasts alters the fate of the incident light, which passes through the leaf without encountering excess absorbents. As a result, the proportion of light transmittance increases rather than light absorption (Parry et al., 2014). Lowering light absorption resulted from 'sieve effect' can lower the efficiency of the PCMs especially at high concentrations of chlorophyll. Sieve effect directly impacts the proportion of light transmittance at the index (SPAD-502+ = 650nm; CCM-200 = 653nm) and reference (SPAD-502+ = 940nm; CCM-200 = 931nm) bands used to calculate SPAD and CCI optic indices in the respective PCMs (Cassol et al., 2008; Dong et al 2019). Lowering the proportion of light absorption at the index band resulted from sieve effect is manifested by lowering the values of the optic indices derived from the PCMs despite the higher Chl_{area} recorded *in vitro*. Consequently, for a given PCMs reading, Khalas cultivar yielded the highest Chl_{area}; whereas, Khunaizi yielded the lowest. On the other hand, Barhi and Hilali yielded similar Chl_{area}, which ranged between Khalas and Khunaizi predicted Chl_{area}.

In addition to the above, and similar to the findings of Pal et al. (2012), the generic species-specific models exhibit a tendency to underestimate the Chl_{area} in the leaflets of cultivars with high chlorophyll concentration, such as Khalas. This points out the limitation of the generic species-specific models used to estimate Chl_{area} in date palm leaflets. Nevertheless, the generic prediction models constructed to illustrate the relationship between the pooled Chl_{area} in date palm leaflets and PCMs optic indices are very strong as they recorded high coefficient of determinations along with low relative errors. This confirms the validity of using the generic species-specific fits for rapid and nondestructive estimation of chlorophyll concentration in date palm leaflets. Similarly, Jifon et al. (2005), reported variations in the relationships between SPAD-502+ and CCM-200 reading and actual chlorophyll concentration of individual citrus cultivars, suggested the possibility of obtaining a single prediction equation for the investigated citrus cultivars. The derived generic functions can have many eminent applications. It can greatly support the work of both researchers and practitioners alike under conditions where constructing cultivar specific calibration fit is constrained. Time and effort needed for calibrating SPAD-502+ and CCM-200 for individual cultivar would be significantly reduced by using the generic species-specific functions to estimate the Chl_{area} in date palm leaflets (Dong et al., 2019).

Both SPAD-502+ and CCM-200 are light transmittance based PCMs. They measure the transmission of two wavelengths of light through plant leaves (red: 650nm and 653nm and near infrared (NIR): 940nm and 931nm; respectively) (Parry et al., 2014; Dong et al., 2019). The differences denoted between the performances of the two PCMs were attributed to the equations used to calculate the relative optic indices (SPAD and CCI). SPAD-502+ uses logarithmic equations that includes two proprietary constants defined only by the manufacturer.

In contrary, CCM-200 uses simple equation that calculates CCI based on the ratio of light transmission at NIR and Red wavelength. Besides, SPAD-502+ sensing area is smaller 6 mm^2 compared to CCM-200 71 mm^2. The larger sensing area of CCM-200 provides a wider spatial measure of chlorophyll in date palm leaflet compared to SPAD-502+. However, the wider sensing area has the tendency to increase the heterogeneity of chlorophyll distribution and hence increases the variability in CCM-200 readings compared to SPAD-502+ (Parry et al., 2014). The aforementioned suggest that SPAD-502+ may have the tendency to minimize the effect of the internal leaflet structure and chlorophyll heterogeneity on the accuracy of chlorophyll estimation compared to CCM-200 (Dong et al 2019). However, the R^2 values and the Error % of the prediction functions derived for both PCMs did not support this argument and indicated that the two PCMs provide equally accurate chlorophyll estimates. In addition, SPAD-502+ and CCM-200 pooled indices demonstrated strong relationships, signifying the validity of exploiting the two devised interchangeably to estimate chlorophyll concentration in date palm leaflets. This finding has significant applications, especially in the field. Date palms are characterized by lanceolate leaflets, which are obliquely attached to the frond petiole. Leaflets blades are folded longitudinally along a large mid vein (Zaid and de Wet, 1999). For accurate measurements, the large mid vein that separates the leaflets into two narrow blades, must be avoided. Therefore, SPAD with narrow sensing area can be used to estimate Chl_{area} in narrow leaflets and narrow tips of the leaflets. On the other hand, CCM-200, which acquire wider sensing area can be employed to measure the average of larger special Chl_{area} of leaflets with wider breadth (Parry et al., 2014).

CONCLUSION

The current study confirmed the validity of exploiting SPAD-502+ and CCM-200 light-transmittance based PCMs, for rapid and nondestructive estimation of total chlorophyll concentration in date palm leaflets. Accordingly, both SPAD-502+ and CCM-200 PCMs can be considered as potential tools for monitoring the physiological status and assessing the health of date palm trees under various environmental conditions. The calibration models derived for the four distinct cultivars indicated that the relationships between Chl_{area} and SPAD and CCI optic indices is cultivar specific. Hence, for precise estimation of chlorophyll concentration, calibration models should be constructed for individual cultivars. Nevertheless, the species-specific models constructed in this study to illustrate the relationship between the pooled data of the four cultivars exhibited strong relationships, hence they can be exploited for generic estimation of chlorophyll concentration in date palm as an independent plant species. The generic conversion equation derived for SPAD and CCI optic indices indicates that the two PCMs can be used interchangeably to estimate chlorophyll concentration in date palm leaflets.

Recommendations

The main recommendations can be summarized as follow:

1- Expanding the range of the investigated cultivars of date palm to assure the accuracy of the species-specific calibration equation used to estimate chlorophyll concentration in date palm as an independent plant species.
2- Investigating the potential of the PCMs for estimating total chlorophyll concentration in leaflets of different developmental stages to minimize the variability among the studied samples.
3- Investigating the possibility of constructing calibration equation to estimate the concentration of Chl_a, Chl_b, carotenoids as well as leaflets nitrogen content using SPAD-502+ and CCM-200.
4- Conducting *in situ* investigation to assess the impact of field conditions on the accuracy of chlorophyll estimation using the studied optical devices.

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Author contributions

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Majeda Salman: Performing the statistical analysis, Drafting the results, Writing - Review and Editing.

Mariam Aljazeri: Methodology, Investigation.

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