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FIELD EVALUATION OF THE RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT IN BASIL LEAVES AND A PORTABLE CHLOROPHYLL METER (SPAD-502) READINGS

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FIELD EVALUATION OF THE RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT IN BASIL LEAVES AND A PORTABLE CHLOROPHYLL METER (SPAD-502) READINGS

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□ *A hand-held SPAD-502 chlorophyll meter provides rapid and nondestructive measures of chlorophyll content. Integrating this meter into basil production can reduce costs and may improve basil quality. This study determined the relationship between SPAD-502 and the leaf chlorophyll (total,* a, b*). Over 500 leaf samples were collected from a field study conducted in 2006. Comparisons between chlorophyll contents and meter readings showed that SPAD meter readings were positively correlated to actual chlorophyll content. Regression analysis SPAD readings should be corrected by leaf area. Findings suggest that SPAD meter readings can be used as a tool to improve Basil quality and for assessing the relative chlorophyll content during the growing season.*

Keywords: photosynthesis, linear regression, chlorophyll, *Ocimum basilicum*, SPAD-502

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INTRODUCTION

Photosynthesis is the most important biochemical process occurring in plants and chlorophyll is the key pigment involved in it (Samdur et al., 2000). In photosynthesis, antenna pigments in leaf chloroplasts absorb solar radiation, and through resonance transfer the resulting excitation is channeled to the reaction centre pigments, which release electrons and set in motion the photochemical process (Richardson et al., 2002). The chlorophylls, Chl *a* and Chl *b*, are virtually essential pigments for the conversion of light energy to stored chemical energy (Gitelson et al., 2003). The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production (Curran et al., 1990; Filella et al., 1995; Ma et al., 1995). From a physiological perspective, leaf Chl content is, therefore, a parameter of significant interest in its own right. Thus, Chl gives and indirect estimation of the nutrient because much of nitrogen is incorporated in chlorophyll (Filella et al., 1995; Hoel and Solhaug, 1998; Samdur et al., 2000; Moran et al., 2000; Hussain et al., 2000; Singh et al., 2002; Giunta et al., 2002; Wang et al., 2004; van den Berg and Perkins, 2004). The concentration of pigments can change with low temperature (Xian-Xiang and Vergara, 1986; Larcher, 2003), ozone injury (Tenga et al., 1989), salinity (Belkhodja et al., 1994; Misra et al., 2001). Pigments can also be used to monitor senescence process and postharvest conservation (Hendry, 1987; Cantwell and Reid, 1994; Philosoph-Hadas et al., 1994; Merzlyak and Gitelson, 1995; Meir et al., 1997; Peñuelas and Filella, 1988). Senescence of the tissues is a result of the oxidation of many compounds, like proteins, lipids and chlorophyll (da Silva et al., 2005).

Fresh basil (*Ocimum basilicum* L.) is a crop where the quality declines with storage. In addition, it has applications as a phytotherapic and therapeutic industries (Vieira and Simon, 2000; Miele et al., 2001a, 2001b). The therapeutic uses are related to essential oil contained in glands and trichomes (Bahl et al., 2000; Lewinsohn et al., 2000; Nacar and Transi, 2000; Vieira and Simon, 2000; Miele et al., 2001a, 2001b; Gang et al., 2001). Oil content can decrease with storage (da Silva et al., 2005), besides, senescence of leaves is frequently followed by an increase of activity of the peroxidases and polyphenoloxidases enzymes, which are responsible for darkening tissues (Underhill and Critchley, 1995).

Considering that senescence has a direct relationship with on leaf chlorophyll content, it is important with basil producers and consumers to know the chlorophyll content before and after harvest. Chlorophyll is measured using laboratory techniques (Richardson et al., 2002). Laboratory analysis is destructive, time-consuming, and expensive. Inexpensive quick methods are needed (Inskeep and Bloom, 1985). It is also destructive, which limits its use

as a diagnostic tool in germplasm screening for higher chlorophyll content or its use in leafy herbs.

Nondestructive optical methods, based on the absorbance and/or reflectance of light by the intact leaf, have been developed and used recently (Richardson et al., 2002). Optical methods generally yield a chlorophyll index value that express relative chlorophyll content but not absolute *Chl* content per unit leaf area, or concentration per gram of leaf tissue. These newer methods are non-destructive, inexpensive, quick and now possible in the field (Buschmann and Nagel, 1993; Gitelson and Merzlyak, 1994a, 1994b; Markwell et al., 1995; Gamon and Surfus, 1999; Gitelson et al., 2001, 2002) and have been demonstrated to predict chlorophyll content for different species of plants (Yadava, 1986; Marquard and Tipton, 1987; Campbell et al., 1990; Dwyer et al., 1991; Fanizza et al., 1991; Gratani, 1992; Monje and Bugbee, 1992; Markwell et al., 1995; Castelli et al., 1996; Hoel and Solhaug, 1998; Azia and Stewart, 2001; Yamamoto et al., 2002; Zotarelli et al., 2002; Richardson et al., 2002; Cate and Perkins, 2003; Gitelson et al., 2003; Murillo-Amador et al., 2004; Wang et al., 2005; Uddling et al., 2007). After establishing a general correlative relationship for a particular species, it is possible to use a chlorophyll meter in applications for which precise values are not necessary. For example, rapid assessment of relative chlorophyll in basil would be of particular utility for basil producers or researchers investigating the senescence, the oil content, the symptoms of which include foliar nutrient deficiencies and reduced chlorophyll content. The accuracy and utility of the handheld (SPAD-502) chlorophyll meter for rapid and predicting chlorophyll content in basil leaves has not been previously reported. However, differences between regression equations for chlorophyll content and SPAD index in some crops has been reported (Campbell et al., 1990; Fanizza et al., 1991; Yamamoto et al., 2002). This difference might have been partly due to the differences in specific leaf weight (SLW), one of indicator of leaf thickness (Yamamoto et al., 2002). Thus, the objective of this study was to establish the ability of a portable chlorophyll meter (SPAD-502) to estimate total chlorophyll (Chl a + Chl b) in basil and correlates these data with extractable chlorophyll obtained by a conventional method. Moreover, this study discusses the influence of SLW and SPAD readings in chlorophyll basil leaves.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted under field conditions at La Paz-El Carrizal valley, located in a semiarid zone of the state of Baja California Sur, northwestern Mexico, 60 km south of La Paz (23°43′ N, 110°10′ W),

approximately 320 m above sea level. The area receives on average 150 mm yr^{-1} rainfall, about 90% of which occurs from July to September. Mean, maximum, and minimum temperatures are 19.1, 31.6, and 9.4°C during basil cropping (January to August) respectively. Meteorological observations during the study were obtained from an automated weather station. The experimental site has a semiarid climate and sustaining a xerofitic vegetation, there are soils with A-AC-C profiles, classified as Haplic Regosols (Eutric) (Arenic) (IUSS Working Group WRB, 2006) or Entisols (Soil Taxonomy). The soils are characterized by good conditions of aeration and penetrability for plant roots and a low-medium water retention, which tends to be a disadvantage in a semiarid zone. There are low reserves of all macronutrients. Nevertheless, the availability of N and P depends on the microbial activity to transform soil organic matter. Soil exhibits favorable site characteristics for vegetation and organic agriculture, which are limited by the regional climatic conditions.

Crop Management and Plant Material

The land was prepared by two crisscross plowings and two harrowing. Land was leveled and furrowed for basil transplanting on December 2005. Thereafter, 25-d-old seedlings were transplanted at 30 cm spacing between plants and 80 cm between furrows, with a plant density of 62,000 plants ha^{-1}. We used leaves from basil (*Ocimum basilicum* L.) plants cv. 'Nufar' (green-leafed), which were adequate fertilized, irrigated and protected from pests and diseases. All plants were approximately three months old when chlorophyll determinations were made.

Hand-Held Chlorophyll Meter

A hand-help SPAD-502 (Minolta Camera Co., Osaka, Japan) was used to estimate chlorophyll content, which was designed and produced based on Inada's finding (Inada, 1963). This instrument weighs 225 g, has a 0.06-cm^2 measurement area, and calculates an index in SPAD units. The principle of measurement of the SPAD is base on the difference in light attenuation at 650 and 940 nm. The transmittance at 940 nm functions as a reference to compensate leaf variables while the 650 nm source is sensitive to chlorophyll concentration. From the difference in light attenuation a dimensionless SPAD unit, ranging from 0 to 80, is calculated by the microprocessor in the SPAD-502 chlorophyll meter (Azia and Stewart, 2001). The claimed accuracy of the SPAD-502 is $\pm$ 1.0 SPAD units (Richardson et al., 2002). The SPAD makes simple, rapid, and nondestructive measurements to provide a relative indication of leaf chlorophyll concentration compared to the extraction method (Yadava, 1986; Marquard and Tipton, 1987; Yamamoto et al., 2002).

Measurements

Weekly extracted chlorophyll measurements and SPAD readings were taken on the basil plants for five consecutive weeks during July–August. The sampling period in our study, it is a reasonable time for the equation to be used by plant breeders, physiological researchers and for penultimate leaf chlorophyll concentration as measured by SPAD-502 meter to remain stable (Dwyer et al., 1995). Leaf samples were collected between 08:00 and 10:00 h. Two adjacent youngest, fully expanded, healthy, turgid, flat, and homogeneous in color and size leaves were selected, making 1000 in all (200 leaves per week). Five hundred leaves were used for both, field (SPAD) and laboratory chlorophyll (extractable *Chl*) determination and the other 500 leaves were used for specific leaf weight (SLW) determination.

SPAD-Readings

After cleaning surface dust from the selected leaves, on each leaf, three SPAD readings were taken on each side of the midrib. The six readings (36 mm^2 total measurement area) per leaf were averaged to produce a single observation value for each leaf. This procedure was followed for averaging heterogeneity of chlorophyll distribution in the leaf surface (MacNicol et al., 1976). Before taking individual measurements, the meter was set to zero without any sample in the sample box slot by pressing the same button that is used for data collection. While recording SPAD readings, care was taken to ensure that SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs was avoided.

Chlorophyll Extraction

Immediately following SPAD measurement, leaf area was measured with a LI-COR (LI-3000A, LI-COR, Lincoln, NE, USA) and later, three disks from the same leaves (total area of 3.90 cm^2) were sampled using a hole punch, immediately pooled, macerated, and homogenized in 20 ml of cold, 80% aqueous acetone, and transported in a portable icebox to the laboratory. Chlorophyll suspensions were kept in the dark for 72 h prior to measuring absorbance with a spectrophotometer (Spectronic Unicom, Cambridge, UK). Before to measuring absorbance, the pigment extracts were centrifuged for 3–5 min in glass tubes to make the extract fully transparent. Absorbance of leaf extracts was measured at 645 nm and 663 nm, and total chlorophyll content (Chl a + Chl b) was determined by the method of Arnon (Arnon, 1949) and expressed on both, fresh weight (mg g^{-1}) and leaf area basis (mg cm^{-2}).

Specific Leaf Weight

The leaf area of the remaining 500 collected leaves was measured using the same leaf area meter after which the leaves were oven-dried at 80°C

to constant weight (approximately 48 h) before determining the leaf dry weight. The SLW was calculated as the ratio of leaf area to leaf dry weight.

Statistical Treatment of the Data

Pearson's simple correlation coefficients (r) among variables were determined by the CORR procedure in the SAS system (SAS Institute, Cary, NC, USA). The dependent variables in single and multiple regressions were chlorophyll *a*, *b,* and total obtained by extraction, and the independent variable was SPAD-502 chlorophyll reading for simple regression and SPAD-502 readings and SLW for multiple regressions. From this correlations variables, the simple or multiple regression equation ($Y = a + bx$) was developed using the 95% limits of confidence. Regression analysis for linear effects was also performed using the REG procedure in the SAS system, where F, coefficient of determination R^2, P, mean, coefficient of variation, and standard deviation for each dependent and independent variable were obtained. All figures were done with Statistica 7.0 for Windows (StatSoft, Inc., Tulsa, OK, USA). When we detected outliers, which by definition are typical infrequent observations, the data were winsorized to improve the linear regression models, correlation coefficients, determination coefficients, and the regression line generally. In winsorization, values in an ordered array are replaced by neighboring values (Sokal and Rohlf, 1998).

RESULTS

Actual Chlorophyll Content

The total Chl content when it was expressed on a leaf area basis ranged from 0.0098 to 0.0515 mg cm^{-2}; the average *Chl a* and *Chl b* contents were 0.195 and 0.0044 mg cm^{-2}, respectively. Leaf concentrations (mg g^{-1}) of total Chl ranged from 0.379 to 1.661 mg g^{-1} with average of 0.856 mg g^{-1}; the average Chl *a* and Chl *b* contents were 0.70 and 0.17 mg g^{-1}. There was generally a close linear relationship between Chl*a* and Chl*b* in both cases, when Chl was expressed on a leaf area and on fresh weight ($r = 0.80$, $p = 0.000$, $n = 500$). The Chl*a*: Chl*b* ratio was in the range of 2.07–15.74, with average of 5.12.

Relationships between *Chl* Readings (SPAD-502) and Measured *Chl* content (*a, b,* and *Total*)

SPAD-502 readings were significantly related to extracted chlorophyll (*a*, *b,* and *total*) levels of basil leaves in terms of fresh weight and leaf area basis, during basil cropping. The best fit was a linear relationship. Correlation

TABLE 1 Relationship between extracted leaf chlorophyll (Y) and SPAD-502 readings (X) in basil leaves

Components	Simple regression	Correlation coefficients (r)	Coefficients of determination (R^2)
	Extracted chlorophyll in fresh weight basis (mg/g)		
Chlorophyll *a*	$Y = -0.1080 + 0.0262^*X$	0.61**	0.38**
Chlorophyll *b*	$Y = -0.0327 + 0.0062^*X$	0.37**	0.14**
Total Chlorophyll	$Y = -0.1416 + 0.0324^*X$	0.57**	0.33**
	Extracted chlorophyll in leaf area basis (mg/cm^2)		
Chlorophyll *a*	$Y = -0.0046 + 0.0008^*X$	0.73**	0.54**
Chlorophyll *b*	$Y = -0.0014 + 0.0002^*X$	0.42**	0.17**
Total Chlorophyll	$Y = -0.006 + 0.001^*X$	0.67**	0.45**

**Significant at $P < 0.01$.

coefficients (r) and regression equations between SPAD-502 readings and chlorophyll contents are presented in Table 1. Correlation coefficients between SPAD-502 readings and chlorophyll contents were highly significant at all leaves sampled. In terms of fresh weight basis, the r values between SPAD-502 readings and chlorophyll *a* (0.61**), chlorophyll *b* (0.37**), and total chlorophyll (0.57**) were high, positive and significant. Likewise, in terms of leaf area basis, the r values between SPAD-502 readings and chlorophyll *a* (0.73**), chlorophyll *b* (0.42**), and total chlorophyll (0.67**) were high, positive and significant, indicating closer relationship of these traits with the SPAD-502 readings, i.e. the higher the SPAD-502 readings, the higher the chlorophyll pigments will be and vice versa. The regression lines (Figures 1 and 2) showed that these variables are linearly related with each other. The R^2 values for chlorophyll *a*, *b* and *total* chlorophyll on a fresh weight basis were 0.38**, 0.14**, and 0.33**, respectively. On a leaf area basis, the R^2 values were 0.54**, 0.17**, and 0.45**, for chlorophyll *a*, *b*, and *total* chlorophyll, respectively (Table 1).

Based on that the accuracy of portable chlorophyll meters decreases at high SPAD readings (Monje and Bugbee, 1992; Richardson et al., 2002), we used the natural log transformations of SPAD readings and total chlorophyll values to determine if the correlation coefficients (r) or coefficients of determination (R^2) reported in this experiment could be increased to better explain the variation of the data as a linear model; however, these values were not increased significantly. On the other hand, separate multiple regressions were done with chlorophyll (*a*, *b*, and total) as dependent variables, and SPAD readings and SLW as independent variables. These regressions were significant at $P < 0.01$ and all correlation (r) and determination coefficients (R^2) increased their values compared with those values showed in simple regression equations, being higher when extracted chlorophyll was expressed in leaf area basis (Tables 1 and 2).

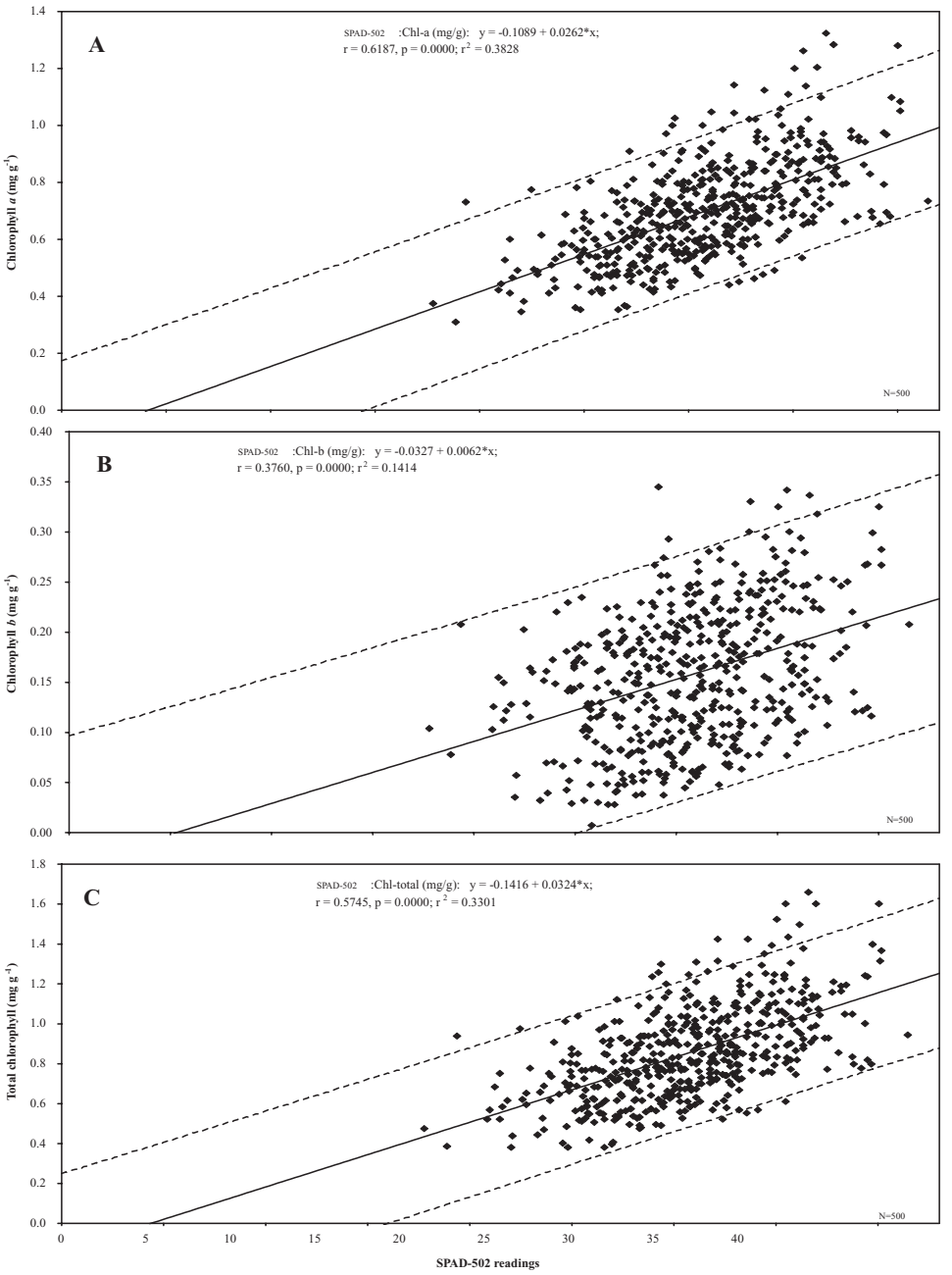

FIGURE 1 Linear regression with 95% prediction limits of the relationship between (A) SPAD readings and chlorophyll *a* (mg/g); (B) SPAD readings and chlorophyll *b* (mg/g); (C) SPAD readings and total chlorophyll (mg/g).

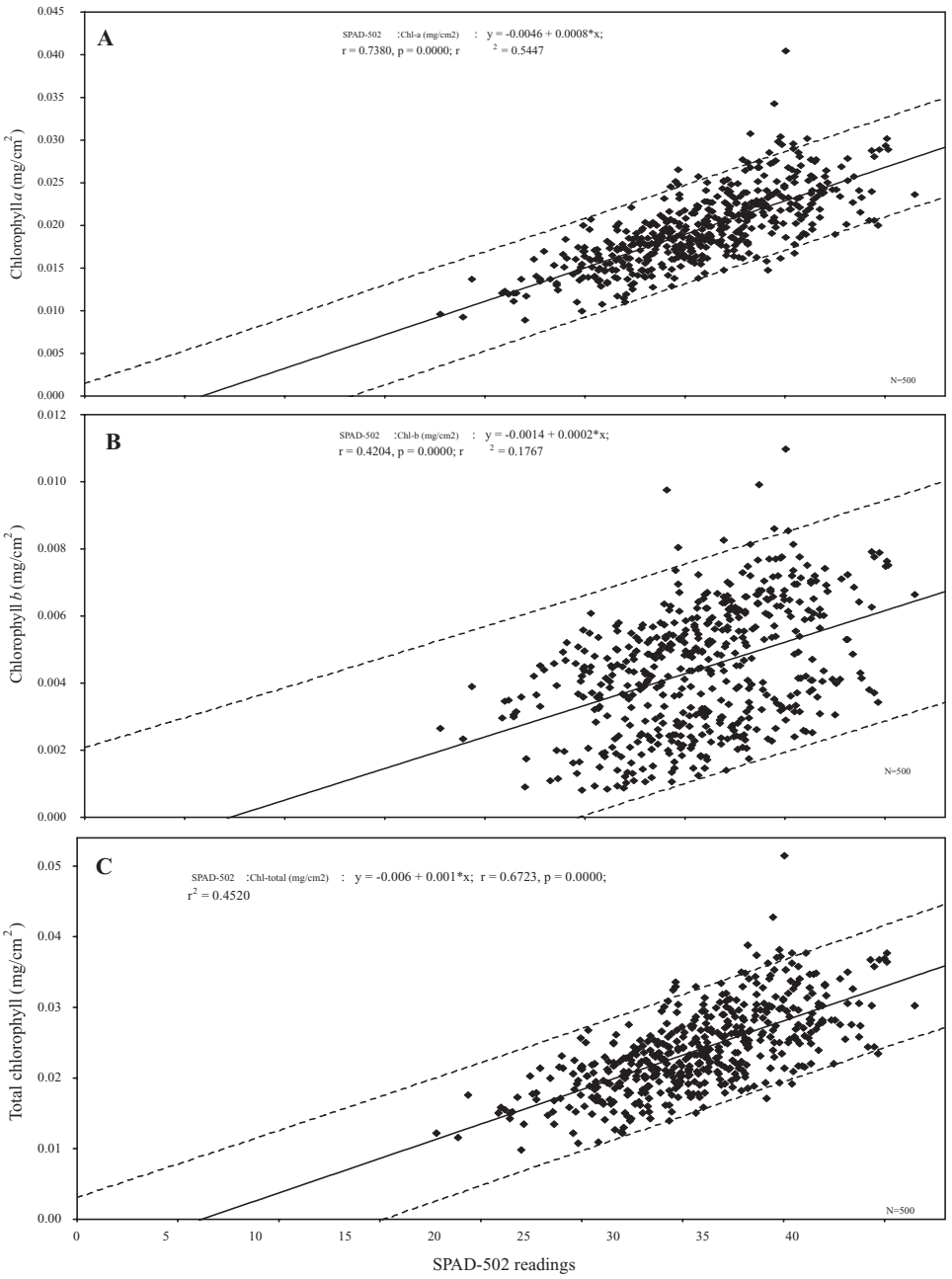

FIGURE 2 Linear regression with 95% prediction limits of the relationship between (A) SPAD readings and chlorophyll *a* (mg/cm^2); (B) SPAD readings and chlorophyll *b* (mg/cm^2); (C) SPAD readings and total chlorophyll (mg/cm^2).

TABLE 2 Relationship between extracted leaf chlorophyll (Y), SPAD-502 readings (X_1) and specific leaf weight (X_2) in basil leaves

Components	Multiple regression	Correlation coefficients (r)	Coefficients of determination (R^2)
	Extracted chlorophyll in fresh weight basis (mg/g)		
Chlorophyll *a*	$Y = -0.1078 + 0.0262^*X_1 - 0.050^*X_2$	0.71**	0.51**
Chlorophyll *b*	$Y = -0.0310 + 0.0072^*X_1 - 0.0260^*X_2$	0.47**	0.22**
Total Chlorophyll	$Y = -0.1366 + 0.0324^*X_1 - 0.0655^*X_2$	0.67**	0.45**
	Extracted chlorophyll in leaf area basis (mg/cm^2)		
Chlorophyll *a*	$Y = -0.0031 + 0.0007^*X_1 - 0.0003^*X_2$	0.74**	0.55**
Chlorophyll *b*	$Y = -0.0007 + 0.0001^*X_1 - 0.0001^*X_2$	0.43**	0.19**
Total Chlorophyll	$Y = -0.0038 + 0.0009^*X_1 - 0.0005^*X_2$	0.68**	0.46**

**Significant at $P < 0.01$. Specific Leaf Weight (SLW) = milligram per square centimeter.

DISCUSSION

The positive relationships found for basil leaves in this study were comparable with most studies in the literature that quantify the relationship between SPAD readings and *Chl* content values employ linear regression. Thus, Yadava (1986) reported a linear relationship between SPAD readings from fresh leaf samples and extracted chlorophyll of 22 different species with pooled r value of 0.843; Xian-Xiang and Vergara (1986) reported a very high r value (0.989) between SPAD reading and chlorophyll content in rice; Marquard and Tipton (1987) in 12 different species found that extractable chlorophyll was significantly related to SPAD readings, and r^2 for individual species ranged from 0.83 to 0.97; Himelrick et al. (1992) reported a linear regression relationship between SPAD-502 readings and extracted chlorophyll of strawberries leaves with a series of r^2 values of 0.93, 0.89, and 0.92 for chlorophyll *a*, Chl *b* and total Chl, respectively; Samdur et al. (2000) in *Arachis hypogaea* found a positive and highly significant correlation between SPAD readings and chlorophyll content with r value of 0.94** for chlorophyll *a*, 0.90** for chlorophyll *b*, and 0.93** for total chlorophyll; Yamamoto et al. (2002) working with sorghum and pigeonpea leaves, found that simple regression of SPAD indices and total chlorophyll content were significant at the 0.1 level, and the correlation coefficients of simple regression were more than 0.90; Murillo-Amador et al. (2004) found a linear relationship between SPAD readings and the extractable leaf chlorophyll content for data pooled from 61 cowpea genotypes ($r = 0.76$). Coefficients of correlation for individual cowpea genotypes ranged from 0.64 to 0.97; Wang et al. (2004) evaluating the feasibility of using SPAD-502 chlorophyll meter for estimating leaf chlorophyll in *Spathyphyllum* Schitt, found significant correlation coefficients between SPAD values and leaf chlorophyll of 0.83, 0.77, and 0.73 for the three cultivars respectively ('Claudia', 'Double Take' and 'Petite'); Wang

et al. (2005) in tropical ornamental foliage plants, highly significantly linear relationships ($r^2 \geq 0.87$) found between SPAD readings and chlorophyll *a*, *b*, or total chlorophyll content.

The correlation coefficients (r) or coefficients of determination (R^2) values reported in basil leaves could be considered lower compared with all species mentioned previously, which apparently reflects a weaker linear relationships between SPAD readings and extractable chlorophyll. However, these values might reflect the different crop response to nitrogen (Politycka and Golcz, 2004) and other nutrient elements (Valenzuela et al., 1994), environmental factors such as carbon dioxide (Sicher, 1998), air temperature (Sorrentino et al., 1997), and light (Demmig-Adams and Adams, 1996). However, the values of chlorophyll content and SPAD readings in basil leaves of this study are equal to those reported in basil by Kopsell et al. (2005), Politycka and Golcz (2004) and da Silva et al. (2005). In the same sense, it is important to considerer that many studies related to chlorophyll content and SPAD readings in different plant species, used in some cases, a reduced number of leaves (Yadava 1986; Marquard and Tipton, 1987; Campbell et al., 1990; Dwyer et al., 1991; Gratani, 1992; Richardson et al., 2002; Cate and Perkins, 2003;van den Berg and Perkins, 2004; Uddling et al., 2007), which according to Little and Hills (1975), when a few observations are used to explain correlations between variables, these conclusions can to conduct a serious errors of interpretation. In the present study, five hundred basil leaves were used to determine the relationship among SPAD readings and chlorophyll content.

When we used the natural log transformations of SPAD readings and total chlorophyll values, the correlation coefficients (r) or coefficients of determination (R^2) values reported here, were not increased such as those reported in sugar maple leaves by Van den Berg and Perkins (2004), who found that this model increased the r^2 value from 0.76 to 0.81. The difference of our results with respect to those reported by van den Berg and Perkins (2004), could be related with the chlorophyll units that those used (μg mm^{-2}), while we reported extracted chlorophyll in milligrams in both cases, on a leaf area basis and a fresh weight basis. Based on this and according with Zar (1999), the logarithmic transformation is especially preferable when some of the observed values are small numbers.

The fact that in the present study a multiple regressions increased the values of correlation and determination coefficients compared with those values showed in simple regression equations, is a evidence and suggest that SLW is an important factor affecting the chlorophyll content (*a*, *b*, and total) on basil leaves, because of SLW is one of the indicators of leaf thickness (Chiariello et al., 1989), which change according to leaf age and environment (Gratani and Bombelli, 2000). Likewise, it has been demonstrated that reflectance increases and transmittance decrease with an increase in leaf thickness (Knapp and Carter, 1998), which is one of the factors that

determines SPAD index under different conditions (kind of crop, growth conditions, and growth stages; Yamamoto et al., 2002). These results are similar those reported by Campbell et al. (1990), Fanizza et al. (1991), Peng et al. (1993) and Yamamoto et al. (2002) who mentioned that another feature reported is the specific leaf weight (SLW), which appears to be one of the factors determining SPAD index under different conditions.

On the other hand, the relationship between SPAD readings and extractable chlorophyll was stronger in both cases, simple and multiple regressions, when it was expressed on a leaf area basis than a fresh weight basis. This result could be related to the wide spread distribution of chlorophyll within the leaf (Azia and Stewart, 2001). Similar results were found when extractable chlorophyll was expressed on a leaf area basis by Xian-Xiang and Vergara (1986) in rice leaves; Marquard and Tipton (1987) in twelve plant species; Tenga et al. (1989) in tomato leaves; Campbell et al. (1990) in apple leaves; Fanizza et al. (1991) in *Vitis vinifera*; Dwyer et al. (1991) in corn leaves; Azia and Stewart (2001) in muskmelon leaves; Yamamoto et al. (2002) in sorghum and pigeonpea; Cate and Perkins (2003) and van den Berg and Perkins (2004) in sugar maple; and Murillo-Amador et al. (2004) in cowpea leaves. This suggests that the SPAD meter might be useful with greater certainty in predicting chlorophyll content in basil leaves if extractable chlorophyll is expressed on a leaf area basis.

Finally, we conclude that the r and R^2 values between SPAD-502 readings and chlorophyll (*a*, *b,* and total) obtained in basil leaves were high, positive and significant, indicating closer relationship of these traits with the SPAD-502 readings, i.e., higher the SPAD-502 readings higher will be the chlorophyll pigments and vice versa. The simple and multiple regression lines showed that these variables are linearly related with each other with higher relationship when chlorophyll was expressed in terms of leaf area basis than when was expressed on fresh weight basis, being higher . However, the multiple regression based on SLW with chlorophyll (*a*, *b*, and total) as dependent variables, and SPAD readings and SLW as independent variables increased and improved this relationship, suggesting that SLW is an important factor affecting the chlorophyll content (*a*, *b*, and total) on basil leaves, because of SLW is one of the indicators of leaf thickness, for which SLW has influence on SPAD-502 readings and causes the different between regression lines for chlorophyll content and SPAD-502 index for which it is necessary to determine it so as to maximize the accuracy of estimating leaf chlorophyll as a function of the SPAD readings. In general terms, the SPAD-502 readings were strongly correlated with chlorophyll content (*a*, *b,* and total) determined by absorbance of extracted pigments, indicating that this instrument can be used in the field to monitor chlorophyll content in basil leaves, and the data confirms too that SPAD is an effective tool for rapid and nondestructive estimation of relative chlorophyll content in basil leaves during the growing season.

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