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Development and Validation of SPAD-502-Based Calibration Models for Estimating Chlorophyll Content in *Axonopus compressus*

Ting Zeng [1,2] and Muhammad Saiful Ahmad-Hamdani [1,3,*]

[1] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; gs64190@student.upm.edu.my
[2] Guangxi Ecological Environment and Landscape Protection Research Center, Guilin Tourism University, Guilin 541004, China
[3] Institute of Plantation Studies, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
* Correspondence: s_ahmad@upm.edu.my

Abstract

Chlorophyll content is an important indicator of turfgrass health, but traditional extraction methods are destructive and time-consuming. This study evaluated the SPAD-502 chlorophyll meter for non-destructive estimation of chlorophyll content in *Axonopus compressus*. Thirty leaf samples were collected from A-40 and A-46, with 15 samples from each variant across five visible leaf color levels. SPAD values were measured in the field, while chlorophyll a, chlorophyll b, and total chlorophyll were determined using 80% acetone extraction and spectrophotometry. SPAD readings showed significant positive correlations with all chlorophyll components in both variants. Five regression models were tested, with strong relationships observed (R^2 = 0.8960–0.9639). Nonlinear models, particularly power and exponential functions, provided better fitting than linear models. A-46 showed slightly stronger relationships than A-40. These results suggest that the SPAD-502 meter can provide a rapid and non-destructive estimate of chlorophyll content in *A. compressus* under the present sampling conditions, supporting its potential use in turfgrass monitoring.

Keywords: photosynthetic pigments; power equation; non-destructive measurement; turfgrass

1. Introduction

Chlorophyll is the main photosynthetic pigment in plants and plays a key role in capturing light for photosynthesis. Its content is often related to photosynthetic capacity, leaf nitrogen status, plant vigor, and stress response [1]. In turfgrass systems, chlorophyll content is also associated with leaf greenness and visual quality, which are important indicators of turf appearance and management performance [2]. In sports and recreational turf, chlorophyll content is not a direct yield trait, but it can serve as a useful physiological indicator of turf color, nitrogen status, stress response, and recovery potential after wear or environmental stress. Therefore, rapid and reliable assessment of chlorophyll content is useful for evaluating turfgrass growth status, nutrient condition, and early physiological changes under field conditions. Traditional chlorophyll determination methods usually involve destructive sampling, followed by solvent extraction and spectrophotometric analysis [3]. Conventional acetone or ethanol extraction protocols require laboratory equipment, chemical reagents, and considerable time for sample processing [4]. These procedures are destructive, labor-intensive, and unsuitable for repeated monitoring of the

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same plants. They also require proper sample preservation and trained personnel, which limits their use in routine turfgrass management under field conditions.

Handheld chlorophyll meters, such as the SPAD-502 meter, provide a rapid and non-destructive approach for estimating chlorophyll status in living plants [5,6]. The device measures leaf transmittance at two wavelengths: 650 nm, where red light is strongly absorbed by chlorophylls, and 940 nm, which serves as a near-infrared reference wavelength. The resulting value is expressed as a dimensionless SPAD reading associated with leaf chlorophyll concentration [7,8]. However, SPAD readings cannot be directly treated as absolute chlorophyll content. Their relationship with extracted chlorophyll concentration may change among species and genotypes, and can also be influenced by leaf thickness, leaf color, pigment distribution, and leaf structure [9]. For this reason, species- or genotype-specific calibration is needed if SPAD values are used to estimate chlorophyll content.

Previous studies have shown high correlation between SPAD value and chlorophyll concentration in major crops such as maize [10], rice [11], wheat [12], and horticultural species such as tomato [13], mango [14], soybean [15] and sugarcane [16]. Compared with crops, turfgrass has received less attention in SPAD calibration studies. SPAD measurement can be difficult in turfgrass, especially in species with narrow and flexible leaves, such as *Cynodon dactylon* and *Zoysia japonica*. The SPAD sensor may not clamp these leaves evenly, and this can reduce the consistency of readings. Calibration is another issue. Equations developed from crops or other turfgrass species may not fit *A. compressus* directly. The SPAD-502 estimates chlorophyll status from light transmitted through the leaf, and this optical signal can be affected by leaf thickness, internal tissue structure, chlorophyll distribution, and other leaf surface or anatomical traits [17]. Previous studies have also shown that the relationship between SPAD readings and actual chlorophyll concentration can vary among species, datasets, and leaf types and that species-specific calibration can improve estimation accuracy [18,19]. For this reason, one SPAD value may not represent the same chlorophyll concentration in different species or genotypes. A separate calibration for *A. compressus* is therefore needed.

A. compressus is a warm-season grass that spreads by stolons. It is often used in tropical and subtropical areas where a low-maintenance ground cover is needed, such as lawns, recreational areas, roadside slopes, erosion-prone sites, and low-input turf surfaces [20]. Apart from turf use, *A. compressus* is also planted to protect exposed soil and help stabilize slopes. Its creeping stems and dense cover allow it to hold the soil surface more effectively [21,22]. It can also occur as a naturalized component of permanent pastures and may be grazed by cattle, sheep, and other herbivores [23]. It has broader leaf blades than many fine-leaved turfgrasses. This makes it easier for the leaf to cover the SPAD-502 sensor window and may reduce light leakage during measurement. Even so, leaf width cannot be the only reason for using SPAD values as chlorophyll estimates. The SPAD reading may also change with leaf texture, leaf thickness, chlorophyll distribution, and growth habit. These traits can differ among species and even among variants of the same species [24]. For A. compressus, chlorophyll assessment is relevant not only for describing leaf greenness, but also for evaluating plant physiological status under turfgrass, ground-cover, and forage-use conditions. For this reason, SPAD readings still need to be calibrated against extracted chlorophyll content. So far, no SPAD-502 calibration model has been reported for A. compressus. Without such a model, SPAD values can only give a rough indication of leaf greenness rather than a reliable chlorophyll estimate.

In this study, two A. compressus variants, A-40 and A-46, were used to develop and compare SPAD-based chlorophyll estimation models. The objectives were to determine the relationships between SPAD readings and chlorophyll a, chlorophyll b, and total chlorophyll content; to compare the performance of linear and nonlinear regression models; and

to identify suitable calibration equations for rapid, non-destructive chlorophyll estimation in A. compressus. We hypothesized that SPAD readings would be positively related to extracted chlorophyll content in both variants, but that the strength of the relationship and the best-fitting regression model would differ between chlorophyll components and between *A. compressus* variants.

2. Materials and Methods

2.1. Plant Materials

The research was conducted at UPM-SATIRI Turfgrass Research Laboratory, Universiti Putra Malaysia (2°59′ N, 101°44′ E), in Serdang, Selangor Malaysia. Two *A. compressus* variants, A-40 and A-46, were used in this study. The two variants were developed from the original *A. compressus* accession through gamma irradiation mutation breeding and were maintained at the UPM-SATIRI Turfgrass Research Laboratory. These plant materials were not collected from the wild. At the time of this study, A-40 and A-46 were maintained as cultivated research materials at UPM-SATIRI. The available New Plant Variety Protection application/reference number is PVBT 049/24, submitted through the Malaysian Intellectual Property Office (MyIPO). No public germplasm database accession number has yet been assigned to these materials. All leaf sampling was conducted in May 2024 during the active vegetative growth stage. The two variants were selected because previous evaluations showed differences in leaf greenness, growth performance, and stress-related traits [25], making them suitable materials for developing SPAD-based chlorophyll calibration models.

2.2. Leaf Color Grading and Sampling Design

Before leaf sampling, leaf color (LC) was evaluated using a TCM 500 NDVI Turf Color Meter (Spectrum® Technologies, Inc., Aurora, IL, USA) to identify canopy areas with different greenness levels. Based on the turf color meter readings and field observation, leaves were grouped into five color levels: yellow-green leaves (T1), light green leaves (T2), medium green leaves (T3), green leaves (T4), and dark green leaves (T5). The turf color meter was used to assist color-zone identification and to reduce subjectivity during leaf color classification. These color levels were used to broaden the range of chlorophyll variation for model calibration, not as formal experimental treatments. For each variant, three leaf samples were randomly collected from different plants within each color level, resulting in 15 samples per variant and 30 samples in total. The use of different plants was intended to improve sampling independence and reduce repeated sampling from the same individual plant. All samples were collected from healthy, fully expanded leaves without visible disease symptoms, mechanical damage, or senescence. For each leaf sample, SPAD readings were taken ten times, and the mean SPAD value was used for subsequent calibration analysis.

2.3. SPAD Measurements

The SPAD values of the samples were measured using a portable chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan) measuring instrument. Measurements were conducted in the field at 8:30 and 9:00 AM. The SPAD chlorophyll meter measures the upper, middle and lower parts of the leaf and obtains the average value of each leaf. All measurements were performed on fully expanded, healthy leaves free from visible damage, disease symptoms, or senescence. Immediately following the SPAD measurement, the measured leaves were collected for subsequent chlorophyll extraction.

2.4. Chlorophyll Extraction and Quantification

Fresh leaf samples were collected immediately after SPAD measurement and transported to the laboratory for pigment extraction. For each sample, 0.1 g of fresh leaf tissue was weighed using an electronic balance. Chlorophyll was extracted with 80% acetone following the method described by Hasan et al. [26], with minor modifications. The samples were ground thoroughly in 80% acetone and kept under low-light conditions during extraction to reduce pigment degradation. The absorbance of the extract was measured at 663.2 and 646.8 nm using a UV-3101PC spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated using the following equations:

$$Chl\ a\ \left(\mathrm{mgL}^{-1}\right) = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8}) \quad (1)$$

$$Chl\ b\ \left(\mathrm{mgL}^{-1}\right) = (21.21 \times A_{646.8}) - (2.79 \times A_{663.2}) \quad (2)$$

$$Total\ Chl\ \left(\mathrm{mgL}^{-1}\right) = (7.15 \times A_{663.2}) + (18.71 \times A_{646.8}) \quad (3)$$

Chlorophyll content was calculated on a sample fresh-weight basis using the following equation:

$$C = \frac{c \times V \times D}{FW} \quad (4)$$

where C is chlorophyll content (mg g^{-1}FW), c is pigment concentration in the extract (mg L^{-1}), V is the extraction volume (L), D is the dilution factor, and FW is the sample fresh weight (g).

2.5. Regression Model Development

SPAD readings were used as the independent variable (x), while chlorophyll a, chlorophyll b, and total chlorophyll contents were used as dependent variables (y). Five regression models were fitted for each pigment and each variant: linear, logarithmic, power, exponential, and quadratic polynomial models. The general equations were as follows: linear, $y = ax + b$; logarithmic, $y = a\ln(x) + b$; power, $y = ax^b$; exponential, $y = ae^{bx}$; and quadratic polynomial, $y = ax^2 + bx + c$. Model fit was assessed using the coefficient of determination (R^2), and candidate models were compared using the Akaike information criterion (AIC). A lower AIC value indicated stronger support after considering model complexity. The selected models were then checked further with LOOCV, RMSE, MAE, and paired *t*-tests.

2.6. Model Validation

To evaluate predictive accuracy, the selected regression model for each pigment and variant was validated using leave-one-out cross-validation (LOOCV). In each validation round, one sample was excluded from the dataset, and the regression model was fitted using the remaining samples. The excluded sample was then predicted using the fitted model. This procedure was repeated until each of the 15 samples for each variant had been used once as the validation sample. The predicted chlorophyll values were then compared with the measured chlorophyll values. Model accuracy was assessed using root mean square error (RMSE) and mean absolute error (MAE). In addition, paired *t*-tests were used to examine whether there were significant differences between measured and predicted values. A non-significant result ($p > 0.05$) indicated no systematic difference between measured and predicted chlorophyll values.

2.7. Statistical Analysis

Descriptive statistics, including mean, standard deviation, and range, were calculated for SPAD readings and chlorophyll content in both variants using R version 4.6.0 and

Microsoft Excel 2024. The relationships between SPAD readings and chlorophyll content were evaluated using five regression models: linear, logarithmic, power, exponential, and quadratic polynomial functions. SPAD readings were used as the independent variable (x), while chlorophyll a, chlorophyll b, and total chlorophyll content were used as dependent variables (y). Figures were prepared using Origin 2021.

3. Results

3.1. Variation in Leaf Color and Chlorophyll Content

Representative leaf color is shown in Figure 1. The progressive darkening of leaf color corresponded closely with increases in chlorophyll accumulation. Yellow green leaf had lowest SPAD value (T1); dark green leaf had highest SPAD values (T5), in both A-40 and A-46, indicating that SPAD shows chlorophyll variation across color levels. Chlorophyll a, chlorophyll b, and total chlorophyll contents increased with SPAD. The visual images of leaf color reflect differences in pigment concentration.

Figure 1. Example of chlorophyll content index in leaves with different shades of green.

Descriptive statistics for SPAD readings and chlorophyll content across the five greenness levels are presented in Table 1. In A-40, SPAD values increased from 15.60 in T1 to 35.20 in T5. A similar increasing trend was observed for chlorophyll pigments. Total chlorophyll content increased from 0.30 mg g^{-1} in T1 to 1.65 mg g^{-1} in T5. In A-46, SPAD values increased from 19.37 (T1) to 39.33 (T5). Total chlorophyll content also increased from 0.58 mg g^{-1} (T1) to 2.05 mg g^{-1} (T5). The A-46 showed higher mean SPAD readings and chlorophyll content than A-40 at most greenness levels, especially in the higher greenness groups.

Table 1. Descriptive statistics of SPAD readings and chlorophyll content across leaf color grades.

Variant	Group	SPAD	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (mg g^{-1})
A-40	T1	15.60 ± 1.44	0.17 ± 0.08	0.12 ± 0.03	0.30 ± 0.11
	T2	27.23 ± 1.50	0.48 ± 0.10	0.24 ± 0.04	0.72 ± 0.14
	T3	29.80 ± 0.66	0.88 ± 0.18	0.35 ± 0.07	1.23 ± 0.25
	T4	32.17 ± 1.35	0.90 ± 0.30	0.35 ± 0.10	1.25 ± 0.41
	T5	35.20 ± 0.66	1.21 ± 0.07	0.44 ± 0.04	1.65 ± 0.09

Table 1. *Cont.*

Variant	Group	SPAD	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (mg g^{-1})
A-46	T1	19.37 ± 7.94	0.39 ± 0.31	0.19 ± 0.10	0.58 ± 0.40
	T2	30.60 ± 0.75	0.86 ± 0.11	0.33 ± 0.03	1.19 ± 0.14
	T3	33.17 ± 1.59	0.91 ± 0.09	0.33 ± 0.01	1.24 ± 0.10
	T4	36.00 ± 0.87	1.09 ± 0.28	0.38 ± 0.08	1.48 ± 0.36
	T5	39.33 ± 2.75	1.52 ± 0.23	0.53 ± 0.08	2.05 ± 0.31

Note: Values are presented as mean ± standard deviation (SD), n = 3. The greenness levels were arranged according to increasing SPAD readings within each variant. Chl a, chlorophyll a; Chl b, chlorophyll b; Total Chl, total chlorophyll.

3.2. Relationship Between SPAD Values and Chlorophyll Content in A-40 and A-46

The regression relationships between SPAD readings and chlorophyll content in A-40 and A-46 are shown in Figure 2 and Table 2. SPAD readings showed significant positive relationships with chlorophyll a, chlorophyll b, and total chlorophyll content in both variants. The relationships between SPAD values and chlorophyll pigments in A-40 are presented in Figure 2a. SPAD value showed significant positive correlations with chlorophyll a, chlorophyll b, and total chlorophyll content ($p < 0.05$). All chlorophyll pigments increased with increasing SPAD readings, although the rate and pattern of increase differed among chlorophyll components.

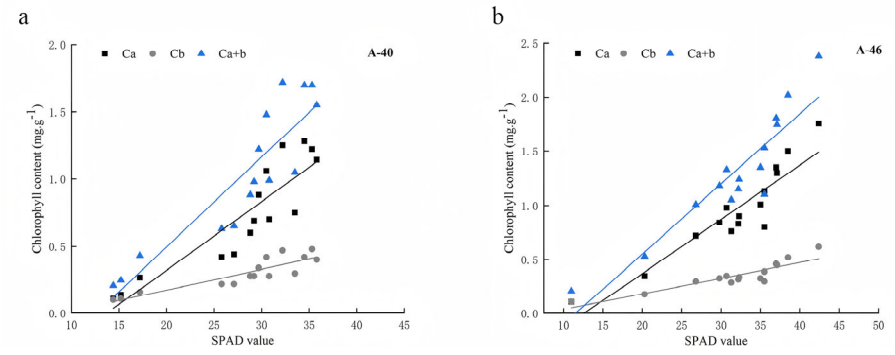

Figure 2. Relationships between SPAD value and chlorophyll a, chlorophyll b, and total chlorophyll contents in A-40 and A-46 leaves. (**a**) A-40; (**b**) A-46.

For A-40, the coefficient of determination for chlorophyll a (R^2) ranged from 0.75 to 0.9248 across models, with the power function ($y = 0.0002x^{2.4861}$) showing the highest goodness of fit ($R^2 = 0.9248$). Similar trends were observed for chlorophyll b ($R^2 = 0.7522$–0.8960) and total chlorophyll ($R^2 = 0.7538$–0.9187). The power and exponential models provided better fits than the linear and logarithmic models in the regression analysis (Table 2).

A-46 showed much stronger SPAD–chlorophyll relationships compared with A-40 in Figure 2b. For chlorophyll a, R^2 values ranged from 0.7362 to 0.9639, with the power model ($y = 0.0008x^{2.0151}$) again producing the highest accuracy ($R^2 = 0.9639$). Chlorophyll b showed good model performance ($R^2 = 0.6902$–0.9219), with the exponential model slightly outperforming other functions. Total chlorophyll showed high correlations across all models, with R^2 values ranging from 0.7272 to 0.9498 (Table 2). Similar to A-40, nonlinear models, particularly the power and exponential functions, generally provided better fits than the linear and logarithmic models. A-46 consistently showed higher R^2 values than A-40 across all chlorophyll components.

Based on AIC, the power model was selected for chlorophyll a and total chlorophyll in A-40, whereas the exponential model was selected for chlorophyll b. In A-46, the exponential model showed the lowest AIC values for chlorophyll a, chlorophyll b, and total chlorophyll. These results suggest that variant-specific calibration equations may be more appropriate than a single general equation for *A. compressus* under the present dataset.

Table 2. Regression equations, R^2 values, and AIC values for the relationships between SPAD-502 readings and chlorophyll content in *A. compressus* variants A-40 and A-46.

Variety	Category	Linear Equation $y = ax + b$	Logarithmic Equation $y = a \ln(x) + b$	Power Equation $y = ax^b$	Exponential Equation $y = ae^{bx}$	Polynomial Equation $y = ax^2 - bx + c$
A-40	Ca	$y = 0.0511x - 0.7017$ $R^2 = 0.8002$	$y = 1.1571\ln(x) - 3.0821$ $R^2 = 0.75$	$y = 0.0002x^{2.4861}$ $R^2 = 0.9248$	$y = 0.03e^{0.1063x}$ $R^2 = 0.9228$	$y = 0.002x^2 - 0.048x + 0.4134$ $R^2 = 0.8416$
	AIC	−48.482	−45.121	−51.816	−51.111	−49.969
	Sig.	*	*	*	*	*
	Cb	$y = 0.0156x - 0.1371$ $R^2 = 0.7901$	$y = 0.3556\ln(x) - 0.8722$ $R^2 = 0.7522$	$y = 0.0017x^{1.5436}$ $R^2 = 0.8906$	$y = 0.0424e^{0.0662x}$ $R^2 = 0.896$	$y = 0.0004x^2 - 0.0057x + 0.1021$ $R^2 = 0.8104$
	AIC	−83.183	−80.696	−84.313	−84.453	−82.706
	Sig.	*	*	*	*	*
	Ca+b	$y = 0.0667x - 0.8388$ $R^2 = 0.8013$	$y = 1.5127\ln(x) - 3.9543$ $R^2 = 0.7538$	$y = 0.0007x^{2.1501}$ $R^2 = 0.9164$	$y = 0.0657e^{0.0921x}$ $R^2 = 0.9187$	$y = 0.0024x^2 - 0.0536x + 0.5155$ $R^2 = 0.8373$
	AIC	−40.606	−37.391	−43.373	−42.984	−41.602
	Sig.	*	*	*	*	*
A-46	Ca	$y = 0.05x - 0.6305$ $R^2 = 0.8513$	$y = 1.0928\ln(x) - 2.7762$ $R^2 = 0.7362$	$Y = 0.0008x^{2.0151}$ $R^2 = 0.9639$	$y = 0.0565e^{0.0845x}$ $R^2 = 0.9348$	$y = 0.0013x^2 - 0.0217x + 0.2172$ $R^2 = 0.9136$
	AIC	−51.540	−42.942	−59.610	−60.254	−57.689
	Sig.	*	*	*	*	*
	Cb	$y = 0.0147x - 0.1132$ $R^2 = 0.8054$	$y = 0.3196\ln(x) - 0.7387$ $R^2 = 0.6902$	$y = 0.005x^{1.2266}$ $R^2 = 0.8936$	$Y = 0.061e^{0.0531x}$ $R^2 = 0.9219$	$y = 0.0004x^2 - 0.0091x + 0.1676$ $R^2 = 0.8803$
	AIC	−83.414	−76.442	−87.599	−92.085	−88.706
	Sig.	*	*	*	*	*
	Ca+b	$y = 0.0647x - 0.7437$ $R^2 = 0.8426$	$y = 1.4124\ln(x) - 3.5149$ $R^2 = 0.7272$	$y = 0.0031x^{1.7304}$ $R^2 = 0.9498$	$y= 0.1134e^{0.0733x}$ $R^2 = 0.9404$	$y = 0.0018x^2 - 0.0308x + 0.3849$ $R^2 = 0.9079$
	AIC	−42.806	−34.556	−50.157	−52.039	−48.847
	Sig.	*	*	*	*	*

Note: In each equation, x represents the SPAD-502 reading and y represents chlorophyll content. AIC, Akaike information criterion. Lower AIC values indicate stronger model support among the candidate models for the same variant and pigment. The asterisk (*) indicates significance at $p < 0.05$.

3.3. Validation of the Prediction Models

To avoid relying only on R^2, model selection and validation were further assessed using Akaike information criterion (AIC), leave-one-out cross-validation (LOOCV), root mean square error (RMSE), mean absolute error (MAE), and paired *t*-tests between measured and SPAD-predicted chlorophyll contents (Table 3). Based on the lowest AIC values, the power model was selected for chlorophyll a and total chlorophyll in A-40, while the exponential model was selected for chlorophyll b. In A-46, the exponential model showed the lowest AIC values for chlorophyll a, chlorophyll b, and total chlorophyll.

The LOOCV results showed relatively low prediction errors for all selected models. In A-40, the RMSE values ranged from 0.0593 to 0.2337 mg g^{-1} FW, and the MAE values ranged from 0.0466 to 0.1950 mg g^{-1} FW. In A-46, the RMSE values ranged from 0.0463 to 0.1709 mg g^{-1} FW, while the MAE values ranged from 0.0354 to 0.1299 mg g^{-1} FW. The paired *t*-test results showed no significant differences between the SPAD-predicted and measured chlorophyll contents for chlorophyll a, chlorophyll b, and total chlorophyll in both A-40 and A-46 ($p > 0.05$). These results indicate that the AIC-selected models showed relatively low prediction errors and no obvious systematic bias within the present calibration dataset.

Table 3. Leave-one-out cross-validation performance of the AIC-selected models for estimating chlorophyll content in A-40 and A-46.

Variants	Pigment	AIC	RMSE	MAE	t Values	*p* Value [a]
A-40	Chl a	−51.816	0.1769	0.1434	0.0283	0.9778
	Chl b	−84.453	0.0593	0.0466	0.0876	0.9315
	Total Chl	−43.373	0.2337	0.1950	−0.0188	0.9853
A-46	Chl a	−60.254	0.1314	0.1046	0.1457	0.8862
	Chl b	−92.085	0.0463	0.0354	−0.2566	0.8013
	Total Chl	−52.039	0.1709	0.1299	0.0195	0.9847

Note: AIC, Akaike information criterion; LOOCV, leave-one-out cross-validation; RMSE, root mean square error; MAE, mean absolute error. RMSE and MAE are expressed in mg g^{-1}. FW and were calculated from LOOCV. Lower AIC values indicate stronger model support among the candidate models for the same variant and pigment. [a] *p* values were obtained from paired *t*-tests between measured and SPAD-predicted chlorophyll contents.

3.4. Comparison of Model Performance Between A-40 and A-46

The calibration performance differed slightly between A-40 and A-46. Based on the regression results, A-46 generally showed higher R^2 values than A-40 for chlorophyll a, chlorophyll b, and total chlorophyll. For chlorophyll a, the highest R^2 value increased from 0.9248 in A-40 to 0.9639 in A-46. For chlorophyll b, the highest R^2 value increased from 0.8960 in A-40 to 0.9219 in A-46. For total chlorophyll, the highest R^2 value increased from 0.9187 in A-40 to 0.9498 in A-46.

The AIC and LOOCV results showed a similar overall pattern. For the selected models, A-46 showed lower AIC values than A-40 for chlorophyll a, chlorophyll b, and total chlorophyll. A-46 also had lower RMSE values than A-40 for all three chlorophyll variables. The RMSE values decreased from 0.1769 to 0.1314 mg g^{-1} FW for chlorophyll a, from 0.0593 to 0.0463 mg g^{-1} FW for chlorophyll b, and from 0.2337 to 0.1709 mg g^{-1} FW for total chlorophyll. The MAE values were also lower in A-46 than in A-40 for all three variables. Together, these results suggest that A-46 showed slightly better calibration performance than A-40 within the present dataset.

4. Discussion

This study showed a clear relationship between SPAD-502 readings and extracted chlorophyll content in *A. compressus*, with the selected AIC-supported models producing R^2 values from 0.8960 to 0.9639. These results suggest that SPAD-502 readings can provide useful estimates of chlorophyll content in *A. compressus* under the present sampling conditions. This needs to be considered because SPAD readings are only relative index values and do not directly measure pigment concentration [27]. Without calibration, one SPAD value may represent different chlorophyll contents in different species [28]. Therefore, the equations developed for A-40 and A-46 make the interpretation of SPAD readings more specific to these two *A. compressus* variants. The LOOCV results also showed acceptable prediction accuracy in both A-40 and A-46, suggesting that this method has potential for rapid and non-destructive chlorophyll monitoring in tropical turfgrass. The R^2 values obtained in this study were comparable to those reported for other grass species and turfgrass systems [29,30]. A previous evaluation across several plant species reported that the strength of the SPAD–chlorophyll relationship varied widely, with R^2 values ranging from 0.02 in wheat to 0.92 in linseed [9]. This helps explain why a separate calibration model was needed for A. compressus. The relatively high R^2 values (>0.89) suggest that the broader leaves of *A. compressus* may be suitable for SPAD-502 measurement, but further anatomical and optical measurements would be needed to confirm the underlying leaf traits affecting this relationship.

Nonlinear models, especially power and exponential functions, generally performed better than linear and logarithmic models in estimating chlorophyll a, chlorophyll b, and total chlorophyll, as supported by both R^2 and AIC results. This result indicates that the SPAD–photosynthetic pigment relationship may be nonlinear, as the SPAD signal is derived from differences in light transmittance at two wavelengths [31,32]. Similar patterns have also been reported in other SPAD calibration studies, where the best model varied among species, pigments, and leaf conditions [33]. Different pigment types may have different optical signatures in leaf tissues [34]. A-46 showed slightly better calibration performance than A-40 within the present dataset, as reflected by higher maximum R^2 values, lower AIC values, and lower LOOCV prediction errors for the selected models. The differences among variants may be caused by leaf anatomical characteristics, including epidermal thickness, mesophyll cell arrangement, chloroplast distribution, and intercellular air spaces, which all influence light transmittance properties and affect SPAD–chlorophyll relationships [35–37]. Previous evaluation of these variants also suggested that A-46 had

relatively stronger leaf growth and chlorophyll-related traits than A-40 [25]. Drawing on the findings of Lhotáková et al. [18], such a discrepancy in model performance may stem from variations in leaf thickness and the vertical distribution of chloroplasts, both of which alter internal light paths and subsequently influence SPAD readings. Therefore, the difference between A-40 and A-46 may reflect variant-related differences in leaf structure and chlorophyll distribution, but further anatomical observation would be needed to confirm this explanation.

In practice, these calibration equations can make field chlorophyll assessment in *A. compressus* more reliable. Once the readings are calibrated, SPAD-502 can be used to estimate leaf chlorophyll status quickly without cutting plant samples. This is practical for turfgrass management, as leaf greenness is linked to turf appearance, plant vigor, fertilization decisions, and stress monitoring [38]. For sports fields, lawns, and other managed turf areas, repeated SPAD measurements can help follow changes in turf condition over time and reduce the need for destructive sampling. These models are also linked to the design of the SPAD-502 meter. The relatively broad leaves of *A. compressus* can cover the sensor window, which helps make the reading more stable. This may not be the case for finer-leaved turfgrasses, where leaf clamping and measurement area can become problems [39]. For such species, a meter with a smaller reading area, such as the MC-100, may be easier to use. The equations in this study should therefore be understood as SPAD-502 calibration models for *A. compressus*, not as a direct comparison among chlorophyll meters. These models were based on A-40 and A-46 samples collected during a single growth period. However, SPAD readings and chlorophyll content may change with environmental conditions, leaf age, nutrient status, water availability, and season. Therefore, the equations should be tested further under different field conditions and management practices before they are used more widely. Chlorophyll content was expressed on a fresh-weight basis in this study because leaf area was not measured for the extracted samples. This makes comparison with studies using chlorophyll per unit leaf area less direct. SPAD-502 readings are taken from the leaf area covered by the sensor window, so leaf-area-based values would be useful in later calibration work. Future studies could include leaf area measurement to make the results easier to compare across studies.

5. Conclusions

This study found that SPAD-502 readings were strongly related to extracted chlorophyll content in *A. compressus*. Of the five regression models tested, the power and exponential models fitted the data better than the linear and logarithmic models. They gave high R^2 values and acceptable LOOCV errors for chlorophyll a, chlorophyll b, and total chlorophyll in both A-40 and A-46. A-46 generally showed slightly higher R^2 values and lower prediction errors than A-40, suggesting that its SPAD–chlorophyll relationship was more stable in this dataset. These results indicate that, with proper calibration, the SPAD-502 meter can be used as a rapid and non-destructive tool to estimate chlorophyll status in *A. compressus*. The equations still need to be tested under different seasons, field conditions, and management practices before they are used more widely.

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