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RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT IN LEAVES OF SORGHUM AND PIGEONPEA DETERMINED BY EXTRACTION METHOD AND BY CHLOROPHYLL METER (SPAD-502)

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ABSTRACT

The estimation of chlorophyll content in leaves by the chlorophyll meter (SPAD 502) is more convenient than by the extraction method for studies on photosynthesis or senescence where the total chlorophyll is estimated on the same leaf over time. This study was to test hypothesis that specific leaf weight (SLW) appears to be one of the factors determining SPAD index

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under different conditions. The influence of SLW on SPAD index and an improved simple method to determine chlorophyll content of sorghum and pigeonpea by a chlorophyll meter was studied. The results indicated that regression lines were significantly different between sorghum and pigeonpea, and at the vegetative and physiological maturity stages in each crop. Residuals of simple regressions calculated from all data of each crop were correlated with SLW. Multiple-regression with SPAD index as the dependent variable, and chlorophyll content and SLW as the independent variables gave the best estimation of chlorophyll content in leaves of sorghum and pigeonpea. These results suggest that SLW is an important factor affecting SPAD index and the influence of SLW on SPAD index can vary with crop species. Devices for estimating SLW could be incorporated into the chlorophyll meter to provide SPAD values adjusted for SLW. Further investigation is required on the influence of SLW on SPAD index for other crops.

INTRODUCTION

The chlorophyll meter (SPAD 502) is a simple, quick, rapid and non-destructive method for estimating chlorophyll content in leaves compared to the extraction method. Estimating the chlorophyll content using the extraction method is laborious, especially when dealing with large number of plants. For studies on photosynthesis or senescence where the total chlorophyll content must be measured on the same leaves over time, the non-destructive method is useful. In this aspect, chlorophyll meter has received much attention for measurement of chlorophyll content.

The chlorophyll meter was designed and produced based on Inada's finding.[1] This instrument determines the relative quantity of chlorophyll present in leaf tissue by measuring the transmittance of the leaf in the red and infrared regions (at a wavelength of about 650 nm and 940 nm, respectively), the latter with self-correction functions. Takebe and Yoneyama[2] reported the regression lines for chlorophyll content and SPAD index were linear and similar among four varieties of rice. They have found only small differences in years and growth stages in the regression lines. The regression lines, however, should be determined for each crop. Castelli et al.[3] have studied winter wheat, maize, soybean and tobacco and found the difference between monocot and dicots bigger than that among monocot or dicot in the regression lines. The regression lines are also different depending on growth conditions and physiological growth stage.

Dwyer et al.[4] has observed differences resulting from different temperature and physiological stages.

In summary, separate linear equations should be developed for each physiological stage, growth condition, species and variety so as to maximize the accuracy of estimating leaf chlorophyll content as a function of SPAD index. Inada[5] has described that the chlorophyll meter reading (SPAD index) is in proportion to chlorophyll content per area for leaves with similar optical properties regardless thickness. Fanizza et al.[6] however, reported some differences between regression equations for chlorophyll content and SPAD index on wine grape cultivars. This difference might have been partly due to the differences in specific leaf weight (SLW), one of indicators of leaf thickness. Does the influence of leaf thickness on SPAD index contribute to better estimation of chlorophyll content by the chlorophyll meter? Leaf thickness changes according to leaf age and environment.[7] Also, it has been demonstrated that reflectance increases and transmittance decrease with an increase in leaf thickness.[8]

Thus, it is hypothesized that leaf thickness is one of the factors that determines SPAD index under different conditions (kind of crop, growth conditions, and growth stages). The lack of a more consistent relationship between chlorophyll concentrations estimated by the extraction method and by the chlorophyll meter for sorghum and pigeonpea at different growth stages, and for different genotypes, limits the potential use of SPAD for these crops. To test this hypothesis, sorghum and pigeonpea were grown in field- or glass house-conditions and the leaves were sampled in different stages.

This study discusses the influence of SLW on SPAD index and improve on the simple method to determine chlorophyll content of sorghum and pigeonpea by the chlorophyll meter.

MATERIALS AND METHODS

Two varieties of sorghum [*Sorghum bicolor* (L.) Moench var. CSH 9 and FSRP] and one variety of pigeonpea [*Cajanus cajan* (L.) Millsp. var. ICPL 87] were used for the measurement of SPAD index, chlorophyll content and specific leaf weight (SLW). Eight leaf tissues with surface area of about $0.002\,m^2$ at vegetative growth stage were taken from each variety of sorghum and pigeonpea grown in a greenhouse at 43 and 54 days after sowing (DAS). The plants were grown in a pot filled with sand at an average temperature of 30°C. Fertilizer was applied at the rate of 2 g N as ammonium sulfate and 0.87 g P as single superphosphate, and water was supplied almost everyday to avoid soil desiccation.

Fifteen samples at middle ripening stage were taken from each variety of sorghum and pigeonpea grown in the Alfisol soil (Ferric Luvisol [FAO]; Udic Rhodustalf [USDA] at ICRISAT Asia center, Patancheru (17°38-N, 78°21-E), India. Fertilizer applied at the rate of 100 kg N ha^{-1} as ammonium sulfate and at the rate of 20 kg P ha^{-1} as single superphosphate.

The SPAD index, chlorophyll content, and SLW were estimated using the same leaf. SPAD index was determined by the average of twenty readings per sample using chlorophyll meter (SPAD-502, Minolta Corp.). The measurement of chlorophyll content was carried out by extraction with ethanol and spectrometer reading at 649 and 665 nm as reported by Shinano et al.[9] SLW was calculated using leaf area and dry weight. Leaf area was calculated from weight of a photocopy of sample leaf and dry weight was measured within subsamples.

The correlation curves were calculated with the minimize square method and significant difference at $P < 0.05$ between the simple regressions formula with analysis of co-variance.

RESULTS AND DISCUSSION

Data were subjected to simple regression analysis, with SPAD index as the dependent variable and chlorophyll content as the independent variable. The results for each variety at respective growth stage are shown in Table 1. All simple regressions were significant at the 0.1% level. The correlation coefficients of simple regressions were more than 0.90, except that for FSRP, the local variety of sorghum. The regression lines were significantly different between sorghum and pigeonpea, and vegetative and middle ripening stages for each crop. However, difference was not observed between two varieties of sorghum at the same growth stages (Table 1). Takebe et al.[2] did not report any difference among varieties of

Table 1. Relationship Between Chlorophyll Concentration ($g\,m^{-2}$) Measured by Method (X) and Chlorophyll Meter (SPAD-502) Reading (Y) in Leaves of Sorghum and Pigeonpea

Crops	Varieties	Growth Stage	Simple Regressions	R^2
Sorghum	FSRP	Vegetative	$Y = 20.8 + 34.7X^{a}$	0.804**
		Ripening	$Y = 13.0 + 55.6X^{b}$	0.578**
	CSH9	Vegetative	$Y = 9.6 + 54.4X^{a}$	0.966**
		Ripening	$Y = 13.3 + 60.3X^{b}$	0.971**
Pigeonpea	ICPL87	Vegetative	$Y = 7.8 + 66.3X^{d}$	0.911**
		Ripening	$Y = 15.2 + 79.4X^{c}$	0.958**

**Significant at $P < 0.01$.
a,b,c,dSignificant difference at $P < 0.05$ between the simple regressions formula followed by different letters.

rice, and they have found small differences between panicle-formation and full heading stages in the regression lines. It may be explained by the similitude of the character in light-transmission through the leaf among varieties of one crop. The difference of crops in simple regressions is caused by different structure of leaves.[3,5] Therefore, we calculated the regression lines separately for each crop. Simple regressions of chlorophyll content and SPAD for each crop were calculated (Fig. 1). The residual of simple regressions was correlated with SLW (Fig. 2). Both the simple regression lines were significant with 0.1% level. This result means the difference on growth stage can be explained by SLW, the thickness of leaves. The slope for pigeonpea was significantly higher than sorghum.

Multiple regression was done with SPAD index as the dependent variable, and chlorophyll content and SLW as the independent variables (Table 2). These regressions for each crop were significant with 0.1% level and coefficients of determination were more than 0.90. The partial coefficients of chlorophyll content for both of crops were similar, around 53. The partial coefficient of SLW for sorghum was 0.15, half of that for pigeonpea.

These results suggested that the effect of chlorophyll content on SPAD index is similar between sorghum and pigeonpea, and that the effect of the SLW on SPAD index is affected by the difference of leaf structure of crops. Using pooled data at diverse growth stages, SLW is an important factor to estimate chlorophyll content using chlorophyll meter. Our results suggest that an additional parameter or function is required for the chlorophyll meter to improve the prediction of SLW, thereby enhancing the accuracy of estimation of chlorophyll content in leaves.

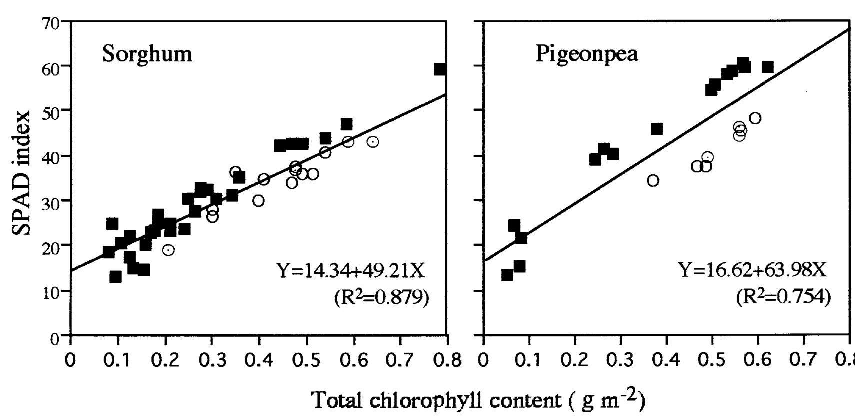

Figure 1. SPAD index on leaves of sorghum and pigeonpea plotted as a chlorophyll content function at vegetative stage (○,⊙: 43, 54 days after sowing) and middle ripening stage (■).

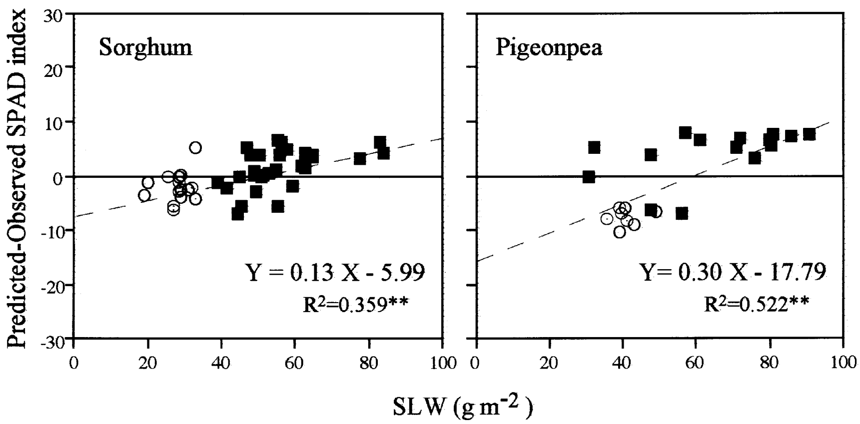

Figure 2. Specific leaf weight (SLW) versus the difference between SPAD index predicted by simple regression curves in Figure 1 and observed SPAD index of sorghum and pigeonpea at middle ripening stage (■) and vegetative stage (○, ⊙: 43, 54 days after sowing). **Significant at $P < 0.01$.

CONCLUSIONS

1. The SLW has influence on SPAD index and causes the difference among growth stages on regression lines for chlorophyll content and SPAD index.
2. The following multiple regression formulas for sorghum and pigeonpea give us better estimation of chlorophyll content using chlorophyll meter with data pooled at diverse growth stages.

$$\text{(sorghum) SPAD} = 6.43 + 52.87 \times \text{Chl} + 0.15 \times \text{SLW}$$

$$\text{(pigeonpea) SPAD} = 4.07 + 53.75 \times \text{Chl} + 0.30 \times \text{SLW}$$

Table 2. Relationships Among Chlorophyll Meter (SPAD-502) Reading (Y), Chlorophyll Concentration Measured by Extraction Method (X_1), and Specific Leaf Weight (X_2) on Sorghum and Pigeonpea

Crops	Multiple Regressions	R^2
Sorghum	$Y = 6.43 + 52.87X_1 + 0.15X_2$	0.924**
Pigeonpea	$Y = 4.07 + 53.75X_1 + 0.30X_2$	0.900**

**Significant at $P < 0.01$.
Unit for chlorophyll content and SLW is gram per square meter.

where Chl is chlorophyll content ($g\,m^{-2}$) and SLW is specific leaf weight ($g\,m^{-2}$).

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