Journal of Agrometeorology 17 (1) : 51-54 (June 2015)

Evaluation and calibration of noninvasive leaf chlorophyll meters for wheat

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ABSTRACT

Nondestructive and fast chlorophyll assessment can be made with portable chlorophyll meters. The meters measure the transmittance of red and near infrared radiation through the leaf and estimates the chlorophyll in non-dimensional index values by defined arithmetic operations.These metes required to calibrate for conversion of index values to the actual chlorophyll content in desired units. Calibration of widely used TYS-A (SPAD meter) and CCM-200 (Chlorophyll content index (CCI) meter) chlorophyll meters wasattempted for chlorophyll estimation in bread wheat (*Triticum aestivum* L.) leaves.The chlorophyll values for the wheat leaves were measured with chlorophyll meters and same samples were used in standard photometric measurements in laboratory. The SPAD values showed statistically significant linear relationship with chlorophyll content (R^2=0.92). The functional relationships represented with regression models can be used to convert the SPAD value to actual chlorophyll content for wheat. The relation between SPAD and CCI was studied using independent leaf samples (n=159). The relationship was found curvilinear and fitted with statistically significant quadratic polynomial (R^2=0.86).So, the fitted quadratic model can be used for inter-conversion between SPAD and CCI. The model was used to convert the values from SPAD to CCI. The derived CCI values were used to fit establish the relationship between CCI and actual chlorophyll content of wheat.

Key words: SPAD, CCI, Chlorophyll meter, wheat, calibration

A measurement of the crop canopy chlorophyll is essential to assess the crop health, nitrogen status, photosynthetic efficiency and stress. The chlorophyll extraction in the laboratory is time consuming and destructive. Besides this, laboratory pigment extraction is tedious for large numbers of samples. So for on-field real time and noninvasive measurements ofchlorophyll, handy chlorophyll meters are preferred mostly. The chlorophyll meters are noninvasive, portable and fast devices to measure the chlorophyll concentration in the canopy of the standing crops/vegetation. These meters measure the transmittance of the red and infrared radiation emitted by LEDs. The transmitted light detected by the sensor as analogue signal and inbuilt mini processor convert this signal into SPAD or index by defined arithmetic operation. The SPAD meters and index meters are widely used for the purposes. These meters provides non dimensional measures (SPAD or index) of the chlorophyll and usually required to convert these measurements either in concentration or content. The functional relationship and close correlation between chlorophyll meter values and extracted total chlorophyll has been reported by many researchers for many crops/ vegetation (Markwell *et al*.1995; Ghasemi *et al*., 2011; Richardson *et al*.,2002; Shaahan *et al*., 1999; Kowalczyk-Jusko and Koscik, 2002; Van den Berg and Perkins, 2004; Chang and Robinson, 2003). These established functional relationships were crop/vegetation specific and cannot be applied universally. So it is essential to calibrate chlorophyll meters for the crops cultivar or species in interest.

This study was conducted to calibrate the TYS-A and CCM-200 chlorophyll meters for bread wheat (*Triticumaestivum L.*) with standard photometric measurements in laboratory.

MATERIALS AND METHODS

The study was conducted on Wheat cultivar GW-496 at Anand (22.538 °N Latitude, 72.981 °E Longitude, 45.1 m MSL) during *rabi* season of year 2012-13.The TYS-A Chlorophyll meter (Zhejiang Top instrument Co., Ltd., Zhejiang, China) and CCM-200 (Opti-sciences) are small and portable chlorophyll meters with almost identical physical build (Fig. 1). Chlorophyll of the canopy can be estimated by clamping the meter over small portion of individual leaves. The TYS-A Chlorophyll meter measures transmittance of 10 mm leaf area at two wavelengths: red

Fig. 1: TYS-A (Zhejiang Top instrument) and CCM-200 (Opti-sciences) chlorophyll meters

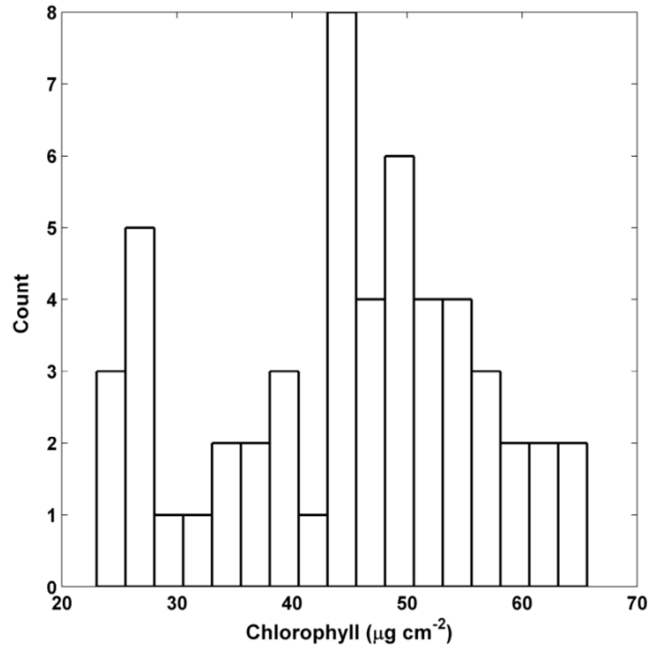

Fig. 2: Frequency distribution of the chlorophyll content of wheat

and near-infrared. Using measured transmittance valuesof red and near infrared the meter computes a non-dimensional SPAD value (0.0 – 99.0) with an accuracy of ±1 SPAD unit. The CCM-200 meter calculates CCI (Chlorophyll Content Index), as ratio of transmittance at 935 nm to 635 nm from specified leaf area of clamped leaf portion.

The leaf samples were collected during year 2012-13 from same experimental wheat plot at different growth stages. The samples were immediately transported to the laboratory for biochemical processing. The leaf samples of 50 mg were prepared using precision weighing balance (Shimadzu Scientific Instruments Inc., USA). To determine chlorophyll concentration per unit leaf area, it was required to measure the leaf area (cm^2) of samples of the 50 mg weight. To measure leaf area, the samples were photographed with a reference with known area and later on photographs converted to binary format and area of the leaf samples calculated by pixel statistics. For each 50 mg leaf sample the mean of 4 SPAD readings were recorded. Laboratory pigments extraction from leaf samples was carried out according to method described by Hiscox and Israelsram (1979). The samples were incubated at 65 °C for 3 hours with 10 ml DMSO (Dimethyl sulfoxide) solvent. The absorbencies at 645 nm and 663 nm were measured with a UV-1700 Pharmaspec (Shimadzu Scientific Instruments Inc., USA) dual beam spectrophotometer.

Total leaf chlorophyll (mg ml^{-1}) calculated using equation proposed by Arnon, 1949.

$$Chl_{a+b}(mgml^{-1}) = 0.0202 \text{ x Abs } 645 + 0.00802 \text{ x Abs } 663$$

Then total leaf chlorophyll converted to ¼g cm^{-2} unit using measured leaf area as:

$$Chl_{a+b}(\mu gcm^{-2}) = \text{Solvent (ml)} \times \frac{Chl_{a+b}}{\text{leaf area }(cm^2)} \times 1000$$

Total of 159 independent leaf samples were collected over different phenophases of wheat crop covering senesced to chlorophyll rich leaves. These samples were used to measure SPAD and CCI values using chlorophyll meters. Approximately same leaf blade portions were chosen to clamp each meter for measurement to achieve maximum precision. Relationship between SPAD and CCI were explored to establish functional relationship for inter-conversion. The inter-conversion coefficients were used to derive CCI values from the SPAD as the CCI measurements were avoided before biochemical pigment analysis to maintain leaf samples fresh, clean and healthy.

RESULTS AND DISCUSSION

SPAD - chlorophyll content relationship

Linear regression was found appropriate to establish the functional relationship between them. Out of 62 set of measurement values, 9 outliers were removed on basis of regression residuals. The frequency distribution of the

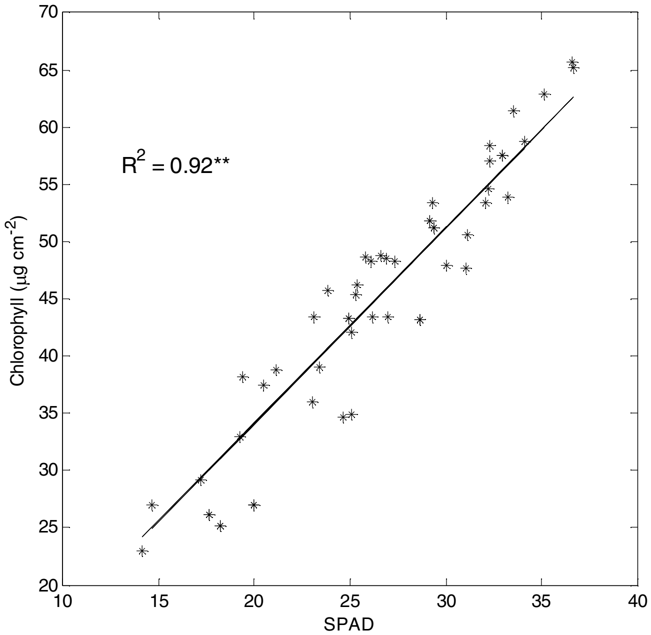

Fig. 3: Linear fit between SPAD and chlorophyll content (n=53)

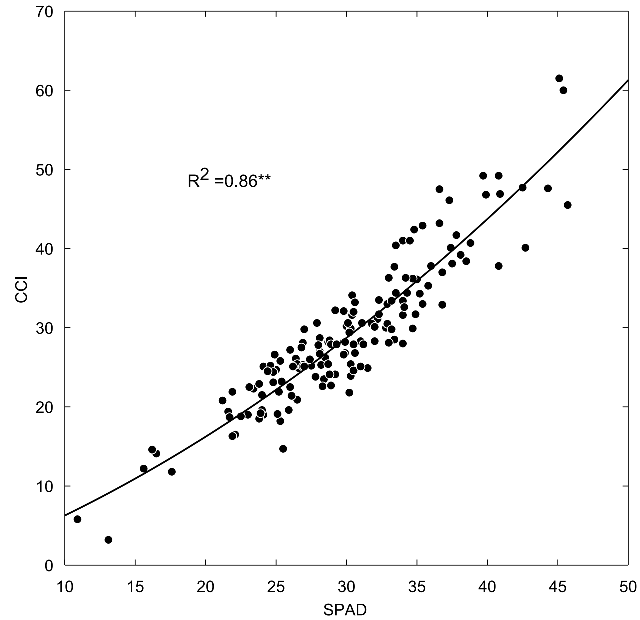

Fig.4: Quadratic fit between SPAD and CCI (n=155)

samples for total chlorophyll content is given in the Fig.2.

The relationships between 53 measurements of SPAD and biochemical chlorophyll a+b extraction depicted in Fig. 3 with scatter plot. There was strong positive relationship between SPAD values and Chlorophyll content. The regression model was found highly significant at 99% level of confidence with coefficient of determination (R^2) of 0.92. Thus, the SPAD values collected from the field can be converted to chlorophyll concentration (¼g cm^{-2}) using relationship as:

$$Chl_{a+b} = 1.7838 \times \text{SPAD} - 1.5333^{**}\ (RMSE = 3.2)$$

SPAD-CCI relationship

The regression analysis was performed on 155 data pairs after removal of 4 outliers affecting the result. The relationship between CCI and SPAD was found curvilinear (Fig.4). The regression analysis revealed that the relationship can be best fitted with quadratic equations. The coefficient of determination (R^2) for the functional relationship between SPAD and CCI was found 0.86. The regression models of the relationship were statistically significant at 99% level of confidence. So, the regression models can be used for inter-conversion between these chlorophyll content indicator values. The inter-conversion model in the form of quadratic equation given below. Other researchers (Knighton and Bugbee, 2002; Richardson *et al.*, 2002) also reported curvilinear relationship between CCI and SPAD.

$$\text{CCI} = 0.013 \times \text{SPAD}^2 + 0.617 \times \text{SPAD} - 1.159^{**}\ (\text{RMSE} = 3.5)$$

CCI-chlorophyll relationship

Assuming SPAD-CCI relationship accurate for conversion of SPAD to CCI, the estimated CCI values were used to derive CCI-chlorophyll relationship for practical purposes. Similar to SPAD- chlorophyll relationship, CCI-chlorophyll relationship was found linear. Ghasemi*et al.*, 2002 also reported linear CCI-chlorophyll relationship for Asian pear tree. The regression model was highly significant with coefficient of determination almost equal to that of SPAD-chlorophyll relation. So, the statistics clearly indicates the usability of this relationship for conversion of CCI values to the chlorophyll concentration (¼g cm^{-2}). The regression model for CCI-Chlorophyll relationship was found as under:

$$Chl_{a+b}(\mu g cm^{-2}) = 2.72 \times \text{CCI} - 32.45^{**}\ (RMSE = 3.7)$$

CONCLUSION

The chlorophyll index values of wheat estimated by portable chlorophyll meters (TYS-A and CCM-200) have statistically significant functional relationship with actual chlorophyll content. So the functional relationship can be useful to calibrate these meters for noninvasive, fast and reliable chlorophyll estimation of wheat crop. The relationship between SPAD and CCI can be exploited for inter-conversion.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the help and support received from Mr. Jignesh Patel, Laboratory Technician and Dr. J G Talati, Professor & Head, Department of Biochemistry, BACA, AAU during laboratory analysis of biochemical leaf pigments extraction.

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Received : June 2014 ; Accepted : December 2014