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Full Length Research Paper

Grain sorghum leaf reflectance and nitrogen status

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Nitrogen deficiency is a common but readily managed constraint to grain yield. A quick and non-destructive detection of crop N status using remote sensing could be a means to increased N use efficiency. Research was conducted in a greenhouse in 2006 at the University of Nebraska-Lincoln to establish the relationship of spectral reflectance with N status in leaves of grain sorghum, to develop indices for interpretation of the results and to predict chlorophyll content. Nitrogen stress decreased chlorophyll meter reading and leaf N content, but increased leaf and canopy reflectance. The SPAD values were significantly increased by both water and N stress. Reciprocal reflectance in the green range (549 to 560 nm), and red edge range (710 to 718 nm) wavelength of the spectrum were good indicators of N stress. The best fit regression between leaf chlorophyll content and the indices in the green and red edge wavebands were linear with an R² of 0.76 to 0.79. A model calibrated using these wavelengths minus reciprocal reflectance of NIR (750 nm), predicted leaf chlorophyll content with root mean square error (RMSE) ranging between 52 and 56 mg m⁻², and reduced the intercept of the model from 312 to 35 mg m⁻² in the green range and 486 to 21 mg m⁻² in the red edge. Future studies will be conducted to evaluate the effectiveness of the indices at the canopy level of grain sorghum.

Key words: Chlorophyll, grain sorghum, nitrogen, red edge, reflectance, SPAD.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is the fifth most important cereal after rice, wheat, maize, and barley. Sorghum is a major food grain for millions in the semi-arid tropics of Africa, Asia, and Latin America and is an important commercial and export crop in the USA, Argentina and Australia (US Grain Council, 2015). Improved varieties respond to N and water stresses as any of the other cereals (Maman et al., 2003; Zhao et al., 2005). It is more tolerant of drought and nutrient stresses

than some other cereals and often well-adapted to semi-arid conditions. Nitrogen availability is often inadequate for optimum grain sorghum yield but lack of available water reduces N uptake and decreases yield response to N (Ferguson, 2000).

Determining N status by remote sensing is one tool for improving N management and yield predictions in many crops. When radiation corresponding to the wavelengths of pigment absorption bands is incident upon green

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vegetation, the reflectance is reduced to a varying extent, depending on the tissue pigment content. Absorption by water and pigments determine to a large extent the reflectance spectrum of a leaf. Chlorophyll and accessory pigments absorb strongly between 400 and 700 nm.

Several studies have reported the use of remote sensing to quantify N stress in many plant species and predict crop yields (Graef and Claupein, 2003; Schlemmer et al., 2005; Gitelson et al., 2005; Zhao et al., 2007). Simple ratio (SR) and normalized difference vegetation index (NDVI) have been widely used to assess ground coverage of plant vegetation, leaf area index (LAI), biomass production and crop yields (Aparicio et al., 2000, Moges et al., 2007). However, other studies have found that NDVI saturates with high biomass and is insensitive to variations in chlorophyll concentration on reaching saturation at low LAI (Aparicio et al., 2000; Daughtry et al., 2000). The use of red-edge reflectance rather than red has improved the early detection of plant stress and crop yield estimation (Gitelson et al., 2005; Eitel et al., 2009; 2011).

Only a few of these indices have been tested on grain sorghum (Mandal et al., 2007; Moges et al., 2007; Zhao et al., 2005). In addition few of these studies considered the combined effect of water and N availability and its effects on leaf reflectance of grain sorghum. Richardson et al. (2002) cautioned that differences in leaf structure may necessitate species-specific calibration equations.

Hypothesis and objectives

Leaf and canopy reflectance of grain sorghum can be used to evaluate N stress. The relationships between leaf and canopy spectral reflectance and water and N stresses of grain sorghum were evaluated. Specific objectives of the study were:

- i. Determine reflectance patterns of sorghum leaf exposed to N and water stress.
- ii. Identify spectral bands in which leaf reflectance were most affected by N content.
- iii. Calibrate and validate spectral indices for the detection of N stress in grain sorghum at leaf level and compare these with published indices.

MATERIALS AND METHODS

The effect of water and N stresses on spectral reflectance of grain sorghum was addressed in a greenhouse study conducted from February to April, 2006. Forty five (45) pots with capacity of 9.45 L were filled with equal volumes of Crete silt loam mixed with sand. Inorganic N as urea was applied at the equivalent of 0, 34, 68, 100 and 135 kg N ha⁻¹ with 50% applied pre-planting and 25% each at 28 and 42 days after emergence to minimize leaching of nitrate-N from the pots. Phosphorus was applied a 45 kg P ha⁻¹ in the form

of triple super-phosphate and potassium at 20 kg K ha⁻¹ in the form of muriate of potash before planting. A medium maturity sorghum hybrid, Dekalb 42-20, was planted and thinned to leave three plants per pot after emergence. A completely randomized design (CRD) with four replications was used. Twelve-hour (7am to 7pm), 400 watt incandescent light was used and temperature was kept at 29°C for day and 18°C for night temperature.

Three levels of soil water matric potential were imposed beginning 32 days after emergence (DAE): adequate/low soil water stress (LOW; <20 kPa); medium water stress (MEDIUM; 40<kPa<80), and high water stress (HIGH; >100 kPa). Soil matric potential was recorded with Watermark sensors (Irrometer Co., Riverside, CA, USA) installed in the middle of each pot. Each Watermark sensor was connected to a data logger and soil water matric potential was logged hourly. At 75 DAE, Minolta SPAD-502 (SPAD) chlorophyll meter, SPAD (Minolta Co., Osaka, Japan) readings were taken from the middle section along the length and midway between the margin and the midrib of the most recently fully expanded leaf. Two measurements were taken from each of three leaves for each pot; afterwards these leaves were removed, kept in a polyethylene bag under ice, and sent to the laboratory for reflectance measurement and chlorophyll extraction (Daughtry and Biehl, 1985). Plants in each pot were harvested, weighed immediately, and then dried at 70°C for 72 h to determine dry weight.

Reflectance measurement, relative water content and chlorophyll extraction

Before chlorophyll extraction, spectral reflectance of the three previously used leaves for SPAD measurement was measured with an ASD Fieldspec FR spectroradiometer (Analytical Spectral Device, Boulder, CO) connected to a Li-COR integrating sphere (LI-COR Inc., Lincoln, NE). A BaSO₄ reference was used to calibrate all reflectance measurements. Six scans were taken per leaf. Each spectral scan measured the reflectance from 350 to 2500 nm at 1-nm increments. The spectral data was converted to reflectance and the data above 2200 nm was discarded due to high noise to signal ratio.

Relative water content (RWC) and total chlorophyll content of the incised leaves were determined. Ten 1 cm disks were taken from each leaf that was used for spectral reflectance measurement. Five disks were selected randomly and weighed immediately providing a measure of fresh weight (L_f). The leaf disks were soaked in deionized water for 24 h and then weighed again to obtain the turgid weight (L_t). Finally, the leaf disks were dried at 85°C and weighed to obtain a dry mass (L_d). The RWC was calculated (Salisbury and Ross, 1992) as:

$$RWC = (L_f - L_d) / (L_t - L_d).$$

The remaining set of five leaf disks were used to determine chlorophyll content using the dimethyl sulphoxide (DMSO) chlorophyll extraction technique (Barnes et al., 1992). Ten milliliters of DMSO and leaf disks were placed in a 65°C water bath for 30 min. The DMSO extract was read on a DU 800 spectrophotometer to acquire absorption (A_λ) measurements at 500 to 750 nm wavelength, which was used to calculate chlorophyll concentration (Chl_{conc}). Equations for Chl *a* and Chl *b* as provided by Wellburn (1994):

$$\begin{aligned} Chl\ a &= 12.19A_{665} - 3.45A_{649}; (\mu g\ ml^{-1}) \\ Chl\ b &= 21.99A_{649} - 5.32A_{665}; (\mu g\ ml^{-1}) \\ Total\ Chl_{conc} &= Chl\ a + Chl\ b; (\mu g\ ml^{-1}) \end{aligned}$$

Table 1. ANOVA summary for a greenhouse study with fertilizer N rates under different levels of soil water stress at 75DAP.

SOV	df	†DMY	Chl	SPAD	RWC	R ₅₄₉₋₅₆₀	R ₇₁₀₋₇₁₈	R ₁₄₅₀₋₁₄₆₀	R ₁₇₆₀₋₁₇₇₀
Units		---g---	mg m ⁻²		-%-	-----%-----			
-----Mean Square(MS)-----									
N rate (N)	4	623**	105063**	363**	0.205**	68.0 **	76.9**	12.8**	8.10**
Water level (W)	2	132**	809.5ns	7.2**	0.084**	20.1**	19.5**	14.8**	10.9**
N*W	8	21.3**	3411**	11.1**	0.014ns	2.78ns	4.61ns	4.95**	3.74**
Residual	28	0.83	377	0.92	0.002	2.45	2.57	1.74	0.81

†Dry matter yield (DMY), total leaf chlorophyll (Chl), SPAD values (SPAD), relative leaf water content (RWC). ‡Reflectance, R at average of 549 to 560, 710 to 718, 1450 to 1460 and 1760 to 1770 nm wavelength. *Significant at 5%, ** Significant at 1% or less, ns Not Significant.

Chlorophyll content was derived as a function of chlorophyll concentration, the volume of DMSO (DMSO_{vol}) used in the extraction, and the leaf disk area (LDA) sampled:

$$\text{Chlorophyll (Chl) content} = (\text{total Chl}_{\text{conc}} * \text{DMSO}_{\text{vol}}) / \text{LDA}; (\text{mg m}^{-2})$$

Indices calibration and validation

Using the concept proposed by Gitelson et al. (2003), four indices were calibrated as follows:

- i. $R_{[\text{green}]}^{-1}$
- ii. $R_{[\text{green}]}^{-1} - R_{[\text{NIR}]}^{-1}$,
- iii. $R_{[\text{RE}]}^{-1}$,
- iv. $R_{[\text{RE}]}^{-1} - R_{[\text{NIR}]}^{-1}$,

Where $R_{[\lambda]}^{-1}$ is the reciprocal reflectance of green, red edge (RE) and near infrared (NIR) and compared with three published frequently used indices listed as follows:

$$\begin{aligned} \text{Simple ratio index (SR)} &= R_{\text{NIR}}/R_{\text{RED}} \text{ (Rouse et al., 1974),} \\ \text{NDVI} &= (R_{\text{NIR}} - R_{\text{RED}})/(R_{\text{NIR}} + R_{\text{RED}}) \text{ (Rouse et al., 1974),} \\ \text{GNDVI} &= (R_{\text{NIR}} - R_{\text{GREEN}})/(R_{\text{NIR}} + R_{\text{GREEN}}) \text{ (Gitelson et al., 1996).} \end{aligned}$$

Data analysis

All data were analyzed by analysis of variance mixed linear model procedure (Proc Mixed, SAS Institute, 2007, Cary, NC, USA). Where the F test was significant at $P \leq 0.05$, the least significant difference (LSD) was calculated and used to separate treatment means. Regression analysis was performed to establish relationships of reflectance with SPAD values, chlorophyll content and biomass yield. The data collected from the experimental setup was divided into two. The data from one set was used to calibrate the reflectance indices and the second data set was used for the indices validation.

RESULTS AND DISCUSSION

Relationship of SPAD values with chlorophyll content and dry matter yield

The interaction between N rate and water-stress levels

significantly influenced dry matter yield, chlorophyll content, SPAD values, and the reflectance mean of 1450 to 1470 nm and mean of 1760 to 1770 nm (Table 1). Water-stress level resulted in significant differences in all parameters measured, except leaf total chlorophyll content.

At each soil water level, the best fit function of the relationship between N rate and biomass yield was a power function with R^2 values of 0.99 with Low and 0.98 with Medium and High water-stress levels (Figure 1A). Water stressed plants responded less to increased N rate and the response to N leveled off after 60 kg N ha⁻¹. Medium and Low water stressed plants reached a plateau between 90 and 120 kg N ha⁻¹. At any N rate, stressed plants had lower yield per kg N applied compared to Medium and Low treatment. Several studies have reported significant water and N interactions on growth and development and biophysical characteristics of crops (Schepers et al., 1996; Martinez and Guiamet, 2004; Zhao et al., 2005).

With an adequate water supply, SPAD values and chlorophyll content had a positive linear relationship with N rate, while the best fit function was power for Medium and High stressed conditions with R^2 values of 0.92 with Medium and 0.98 with High (Figure 1B). With Low water stressed, the relationship between leaf chlorophyll and N rate was linear with R^2 value of 0.97. While with Medium and High, quadratic functions with R^2 values of 0.84 and 0.98 with Medium and High described the relationship between leaf chlorophyll and N rate (Figure 1C).

There was a quadratic relationship between SPAD and chlorophyll content with chlorophyll being increased with increasing SPAD and a coefficient of determination of 0.88 (Figure 2A). Significant relationships between SPAD values and chlorophyll content have been reported by Markwell et al. (1995), Schepers et al. (1996) and Martinez and Guiamet (2004). Since much of leaf N is incorporated in chlorophyll, chlorophyll in leaves has been used to assess N status of crops. Chlorophyll meters have been used to detect N stress in corn (Zea

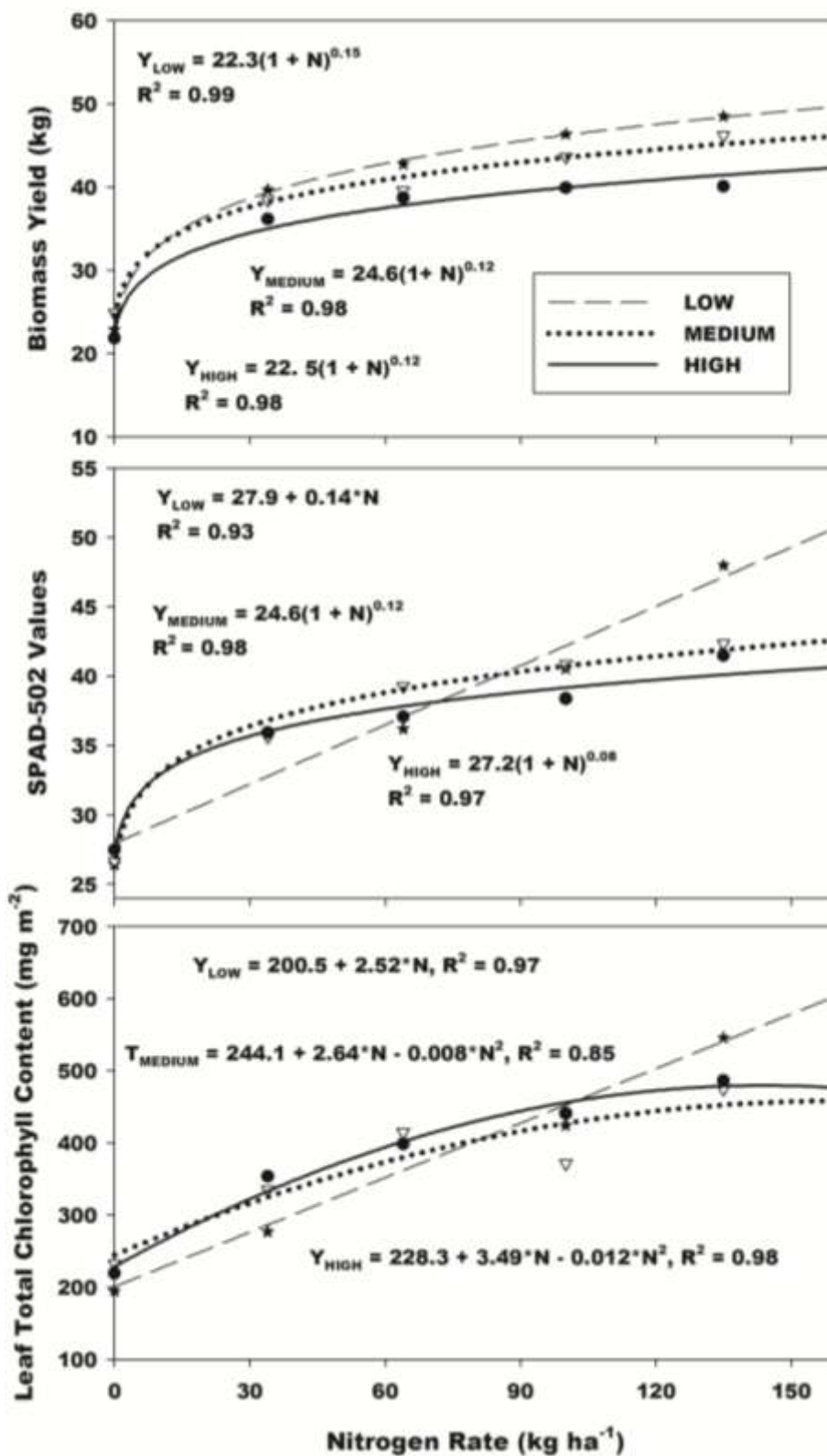


Figure 1. Effect of N rates and water stress levels on grain sorghum biomass yield (A) SPAD values (B) and total chlorophyll concentration (C) at 75 days after emergence in a greenhouse. LOW, MEDIUM and HIGH water stress.

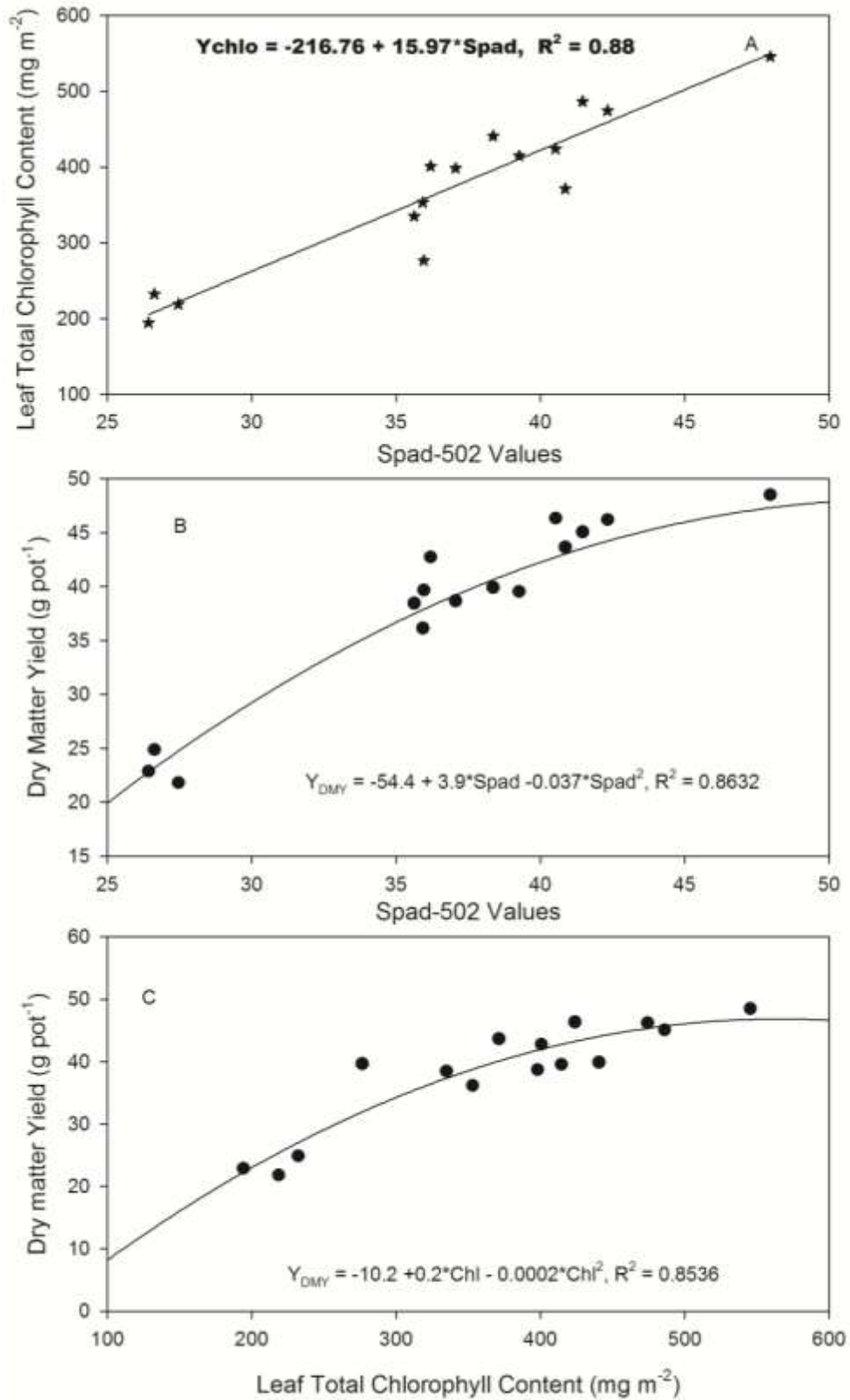


Figure 2. Best fit relationship of SPAD values with grain sorghum leaf total chlorophyll content (Chl) (A) SPAD with dry matter yield (B) and of Chl with dry matter yield (C) across five N rates and three water levels at 75 days after planting in a greenhouse

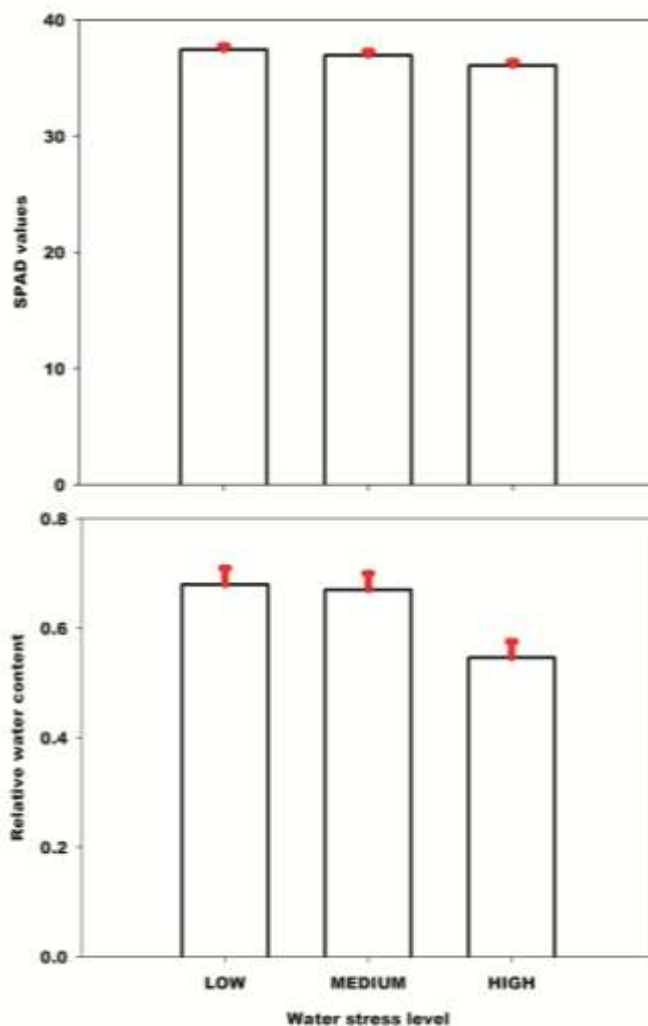


Figure 3. Effect of soil water on leaf SPAD values and relative leaf water content of grain sorghum in a greenhouse study. Y-bars = LSD (0.05).

mays L.) leaves (Schepers et al., 1992; Wood et al., 1992; Blackmer et al., 1994). The increase in dry matter yield relative to SPAD and chlorophyll content was quadratic with R^2 of 0.86 and 0.85, respectively (Figure 2B and C). Although SPAD measurements are rapid and easy, the measurement represents a very small portion of a leaf. Water stress, leaf age and time of the day influence SPAD readings (Schepers et al., 1996; Martinez and Guiamet, 2004; Schlemmer et al., 2005). The use of SPAD in predicting leaf N status in grain sorghum and other plants must be guided by water status of the crop since SPAD tends to under-estimate leaf N status under water stress (Schepers et al., 1996; Schlemmer et al., 2005).

In this study, water stress decreased SPAD values and reduced the relative water content of the leaf (Figure 3).

Relative water content (cell turgor) in plants growing under field conditions varies during the day. Martinez and Guiamet (2004) observed that SPAD values were increased when a maize leaf was dehydrated and reduced when the same leaf was re-hydrated in a laboratory. However, Schepers et al. (1996) and Schlemmer et al. (2005) reported that water stress in maize leaves reduced SPAD values. Water stress in plants reduces RWC and cell turgor and this increases transmittance of the near infrared energy through the leaf tissue. The intercellular air spaces in the leaf tissue are influenced by cell turgor which is directly influenced by plant water status. The SPAD output is a function of leaf transmittance in the red and NIR (650 and 940 nm) wavelengths and is affected by changes in the intercellular air spaces of the leaf.

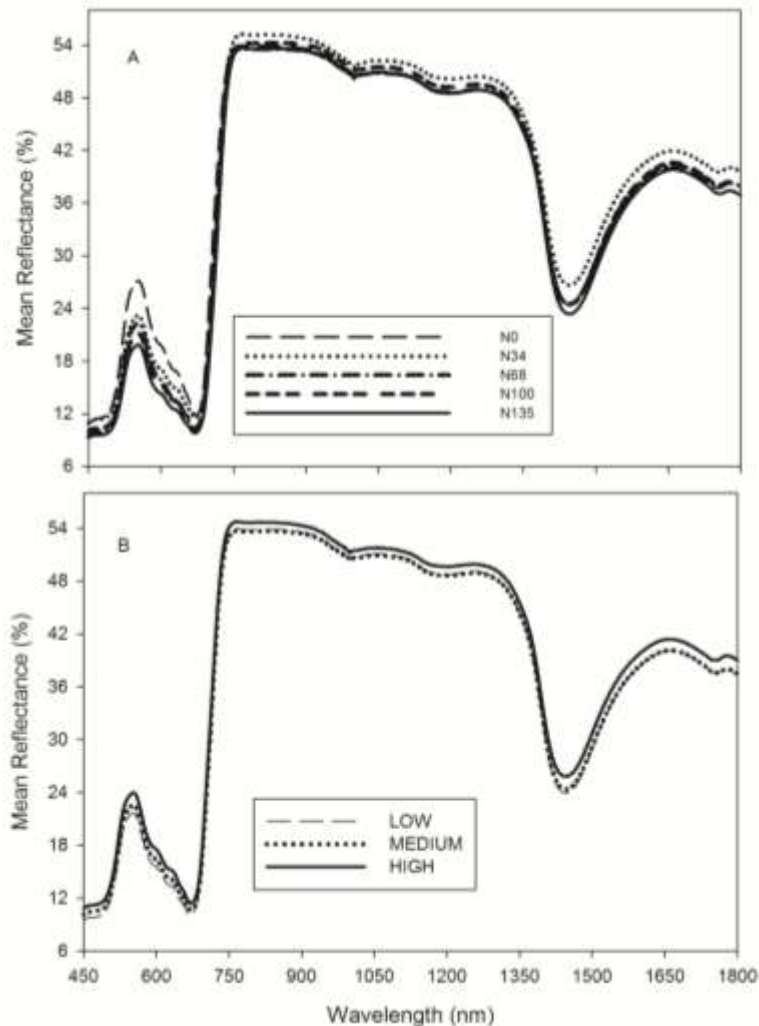


Figure 4. Effect of water (A) and nitrogen (B) stress on leaf spectral reflectance of grain sorghum.

Effect of water and N stresses on leaf spectral reflectance

Water \times N rate interaction resulted in differences in spectral reflectance in the mid-infrared (1450 to 1460 and 1760 to 1770 nm) range (Table 1). In general, inadequate water and N caused increased reflectance in the visible, near infrared (NIR) and mid infrared (MIR) regions of spectral profile for grain sorghum (Figure 4A and B).

In sections of green (549 to 560 nm), red edge (710 to 717 nm) and MIR (1450 to 1460 nm and 1760 to 1770 nm), water and N stress significantly affected the spectral reflectance of sorghum (Table 1). Many environmental and physiological factors can cause increased leaf reflectance, but N deficiency generally increases reflectance in the green and the red edge ranges (Carter and Knapp, 2001; Daughtry et al., 2000; Zhao et al.,

2003). Leaf reflectance in the green, red edge and MIR wavelength of the spectrum can be good indicators of N and water stress in plants (Graef and Claupein, 2003; Schlemmer et al., 2005; Gitelson et al., 2005; Zhao et al., 2007). Reflectance properties of leaves are controlled by the absorption and scattering processes which occur within the leaf. Light is reflected (scattered) at the interface of media with different reflective indexes such as cell wall-air interfaces in the intercellular spaces inside the leaf.

Chlorophyll content was generally high with all N rates (0 to 150 kg N ha⁻¹), ranging from 200 to 500 mg m⁻² (Figure 1C). Variability in chlorophyll content with fertilizer N rate has been observed in other studies on maize and wheat which perhaps are more sensitive to N stress (Schepers et al., 1996; Schlemmer et al., 2005). Sorghum leaf reflectance in the green and red edge

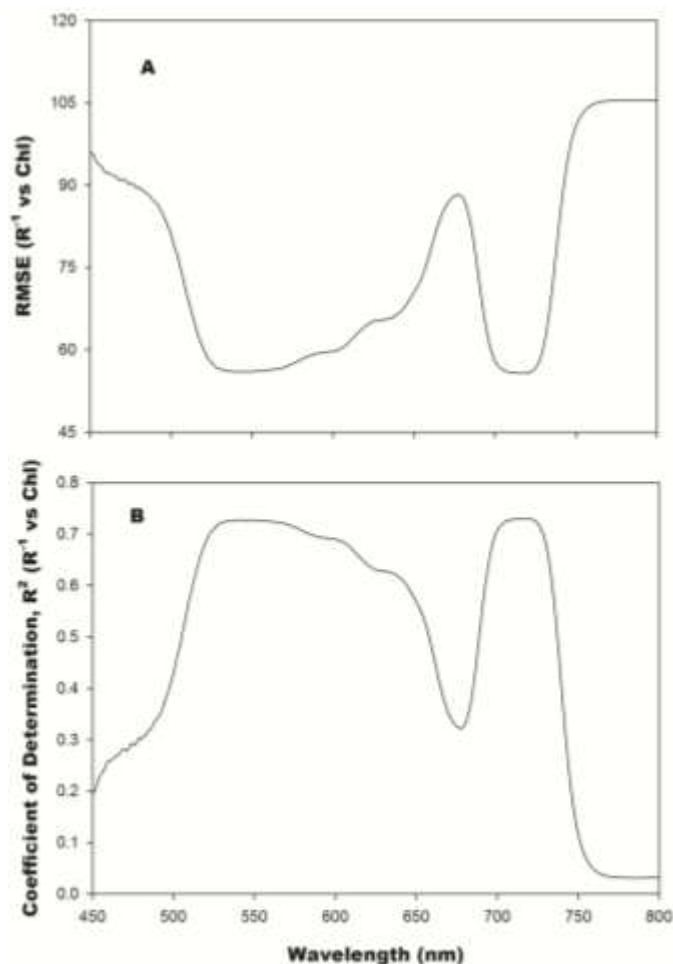


Figure 5. Coefficient of determination, R^2 (A) and root mean square error, RMSE (B) of relationship between reflectance and leaf chlorophyll, and wavelength

wavelengths had the best correlation with chlorophyll content with an R^2 value of 0.74, compared with lower R^2 values of 0.21 in the blue, 0.36 in the red and 0.20 in the NIR wavelength. There is strong absorption of biochemical pigments for photosynthetic activities in the blue and red spectral region. According to Gitelson (personal communication), even in completely yellow leaves, absorption is higher than 85% in the blue spectral region. Due to high reflectance in the green spectral region, the region is sensitive to wide ranges of chlorophyll content, hence the strong coefficient of determination observed. Sims and Gamon (2002) reported that reflectance around the 700 nm spectral region was the most sensitive indicator of chlorophyll of many non-related leaves and that the ratio of NIR to red edge indices proposed by Gitelson and Merzlyak (1994) could be used as a measure of chlorophyll content for many plant species.

Reflectance indices calibration and validation

Using the concept developed by Gitelson et al. (2003), a model was calibrated and validated with an independent data set for leaf chlorophyll content estimation in grain sorghum in a greenhouse study. According to Gitelson et al. (1996), reciprocal reflectance alone at certain wavelengths could be used to quantitatively estimate chlorophyll content. In order to be able to select a spectral range that could be used to calibrate a model for leaf chlorophyll content estimation, a linear correlation between chlorophyll content and spectral reflectance was established. The wavelength with the lowest root mean square error (RMSE) and highest R^2 in the visible and RE regions and the wavelength with the highest RMSE and lowest R^2 in the NIR regions were selected for the calibration (Figure 5). Reciprocal reflectance at 549 to 560 nm ($R_{[549-560]}^{-1}$) with the peak at 550 nm ($R_{[550]}^{-1}$) in

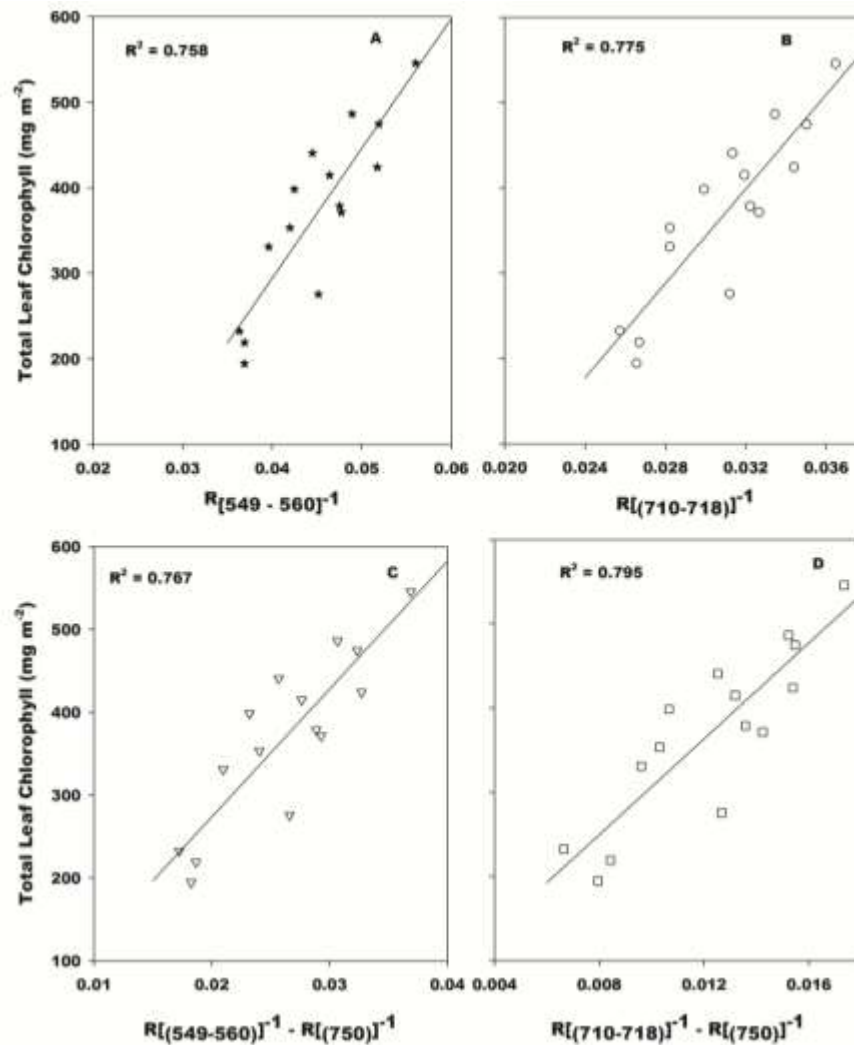


Figure 6. Relationship between reflectance index (R_x)⁻¹ and leaf total chlorophyll content in green (A) and RE (B) spectral range, and subtraction NIR ($R[(750)]$)⁻¹ from (R_x)⁻¹ green (C) and red edge (D).

the green spectral range and from 710 to 718 nm ($R[(710-718)]$)⁻¹ with a peak at 718 nm ($R[(718)]$)⁻¹ in the red edge range were selected for model calibration since this relationship had the highest R^2 and lowest RMSE in the spectral profile and agreed with Gitelson et al. (2003). The best fit regressions between chlorophyll contents and the four reflectance indices were linear with an R^2 of 0.76 to 0.79 (Figure 6).

According to Gitelson et al. (2003), $R[(NIR)]$ ⁻¹ values are comparable to chlorophyll content in leaves with very low chlorophyll content and thus represent scattering and non-pigment leaf absorption. Subtracting $R[(NIR)]$ ⁻¹ values from the green and RE index slightly improved R^2 values and significantly reduced the intercept of the model from 312 to 35 mg m^{-2} in the green range and from 486 to 21 mg m^{-2} in the RE range (Table 2). Zhao et al. (2005)

suggested two narrow ranges centered on $R_{(555)}$ nm and $R_{(715)} (\pm 5)$ nm for detecting N deficiency in sorghum. They found the ratio of two indices R_{1075}/R_{735} and R_{405}/R_{715} had a better linear relationship than single waveband indices with R^2 values ranging from 0.64 to 0.82.

The normalized difference vegetation index (NDVI) and simple ratio (SR) index (Rouse et al., 1974; Aparicio et al., 2000) are two commonly used vegetative indices in remote estimation of chlorophyll in plants. These two indices and the green NDVI (Gitelson et al., 1996) were compared with reciprocal reflectance indices suggested in this study using the same data set. Both NDVI and SR performed poorly, while GNDVI did better compared to the suggested indices in estimating chlorophyll (Figure 7). Gitelson et al. (2003) reported that indices that use

Table 2. Calibrated models for estimating total leaf total chlorophyll (Chl) content in grain sorghum leaves at 75 days after planting in a greenhouse study.

$\dagger R_{\lambda}$	Model	R^2
$R_{[549-560]}^{-1}$	$\text{Chl} = 15176 * R_{\lambda} - 312.78$	0.758
$R_{[549-560]}^{-1} - R_{[750]}^{-1}$	$\text{Chl} = 15426 * R_{\lambda} - 35.154$	0.767
$R_{[710-718]}^{-1}$	$\text{Chl} = 27658 * R_{\lambda} - 486.54$	0.775
$R_{[710-718]}^{-1} - R_{[750]}^{-1}$	$\text{Chl} = 28484 * R_{\lambda} - 21.317$	0.795
SR, R_{780}/R_{685}	$\text{Chl} = 253.1 * R_{\lambda} - 709.67$	0.511
NDVI, $R_{[780-685]}/R_{[780+685]}$	$\text{Chl} = 3768.9 * R_{\lambda} - 1962.4$	0.528
GNDVI, $R_{[780-550]}/R_{[780+550]}$	$\text{Chl} = 2239.8 * R_{\lambda} - 490.8$	0.945

\dagger Reflectance index.

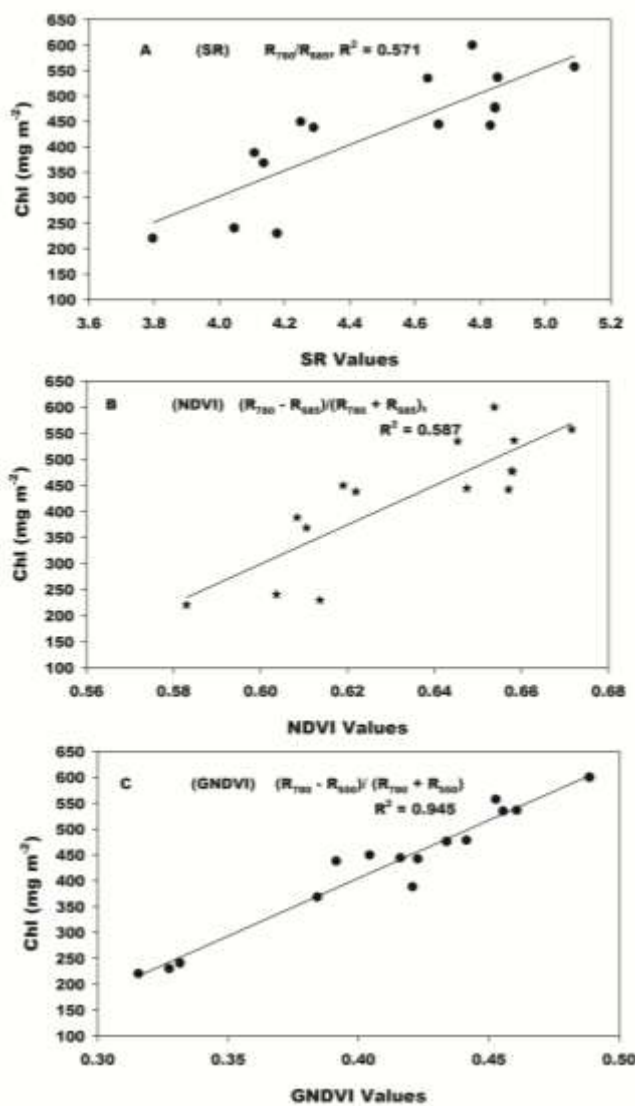


Figure 7. Relationship of total chlorophyll content with reflectance indices: simple ratio (A) normalized difference vegetation index (B) and green normalized difference vegetation index (C).

Table 3. Relationship between predicted and measured total leaf chlorophyll (Chl) content using calibrated models developed from an independent data set.

$^{\dagger}R_{\lambda}$	Model	RMSE (mg m ⁻²)	SE
$R_{[(549-560)]}^{-1}$	$Chl_{pred} = 0.7349 * R_{\lambda} + 107.91$	53.0	48.7
$(R_{[(549-560)]}^{-1} - R_{[(750)]}^{-1})$	$Chl_{pred} = 0.7197 * R_{\lambda} + 112.21$	53.6	48.8
$R_{[(710-718)]}^{-1}$	$Chl_{pred} = 0.7023 * R_{\lambda} + 124.66$	52.4	44.4
$(R_{[(710-718)]}^{-1} - R_{[(750)]}^{-1})$	$Chl_{pred} = 0.6728 * R_{\lambda} + 133.28$	54.0	45.1
SR $[R_{780}/R_{685}]$	$Chl_{pred} = 0.3099 * R_{\lambda} + 262.59$	92.3	67.4
NDVI $[(R_{780} - R_{685})/(R_{780} + R_{685})]$	$Chl_{pred} = 0.3134 * R_{\lambda} + 259.63$	90.4	65.8
GNDVI $[(R_{780} - R_{550})/(R_{780} + R_{550})]$	$Chl_{pred} = 0.7237 * R_{\lambda} + 101.18$	56.0	52.6

† Reflectance index.

reflectance in the red range were sensitive only to low chlorophyll and not sensitive to moderate to high chlorophyll.

Due to the moderate to high chlorophyll of the data set, reflectance in the red spectral region had a low relationship to chlorophyll, and consequently it was not surprising that both NDVI and SR were poorly related to chlorophyll in this study. The calibrated models were used to predict chlorophyll from an independent second data set collected from the same study. Reciprocal reflectance values in the independent data set were used in the indices of the calibrated model to estimate chlorophyll. The estimated chlorophyll was then compared with measured chlorophyll content. Table 3 shows the RMSE and standard error of estimation between the predicted and the measured chlorophyll. The proposed models performed well in predicting chlorophyll, with RMSE ranging from 52 to 56 mg m⁻² (Table 3). As expected both NDVI and SR did poorly with very high RMSE while GNDVI performed better.

Conclusion

With adequate plant water, both SPAD values and chlorophyll content had linear relationships with N application rate. Under water stress conditions, there was the tendency for SPAD values to under-estimate the N status of the plant. Leaf reflectance of grain sorghum was reduced by both N and water stress. Reciprocal reflectance of 549 to 560 nm, $R_{[(549-560)]}^{-1}$ with the peak at 550 nm, $R_{[(550)]}^{-1}$ and 710 to 718 nm, $R_{[(710-718)]}^{-1}$ with peak at 718 nm, $R_{[(718)]}^{-1}$ minus reciprocal reflectance in the NIR, $R_{[(750)]}^{-1}$ had linear relationships with chlorophyll. The models calibrated in this study predicted leaf total chlorophyll with less RMSE than SR, NDVI and GNDVI.

Conflict of interests

The authors have not declared any conflict of interest.

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