



# Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum

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## Abstract

An experiment was conducted under outdoor pot-culture conditions to determine effects of nitrogen (N) deficiency on sorghum growth, physiology, and leaf hyperspectral reflectance properties. Sorghum (cv. DK 44C) was seeded in 360 twelve-litre pots filled with fine sand. All pots were irrigated with half-strength Hoagland's nutrient solution from emergence to 25 days after sowing (DAS). Thereafter, pots were separated into three identical groups and the following treatments were initiated: (1) the control (100% N) continued receiving the half-strength nutrient solution; (2) reduced N to 20% of the control (20% N); and (3) withheld N from the solution (0% N). Photosynthetic rate (Pn), chlorophyll (Chl) and N concentrations, and hyperspectral reflectance of the uppermost, fully expanded leaves were determined at 3- to 4-day-interval from 21 to 58 DAS during the N treatments. Plants were harvested 58 DAS to determine effects of N deficiency on leaf area (LA), biomass accumulation, and partitioning. Nitrogen deficiency significantly reduced LA, leaf Chl content and Pn, resulting in lower biomass production. Decreased leaf Pn due to N deficiency was mainly associated with lower stomatal conductance rather than carboxylation capacity of leaf chemistry. Among plant components of dry weights, leaf dry weight had the greatest and root dry weight had the smallest decrease under N deficiency. Nitrogen-deficit stress mainly increased leaf reflectance at 555 ( $R_{555}$ ) and 715 nm ( $R_{715}$ ) and caused a red-edge shift to shorter wavelength. Leaf N and Chl concentrations were linearly correlated with not only the reflectance ratios of  $R_{405}/R_{715}$  ( $r^2 = 0.68^{***}$ ) and  $R_{1075}/R_{735}$  ( $r^2 = 0.64^{***}$ ), respectively, but also the first derivatives of the reflectance ( $dR/d\lambda$ ) in red edge centered 730 or 740 nm ( $r^2 = 0.73\text{--}0.82^{***}$ ). These specific reflectance ratios or  $dR/d\lambda$  may be used for rapid and non-destructive estimation of sorghum leaf Chl and plant N status.

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**Keywords:** Sorghum; Nitrogen deficiency; Leaf N and chlorophyll; Photosynthesis; Leaf reflectance; Reflectance ratios; Plant N monitoring

## 1. Introduction

Although sorghum [*Sorghum bicolor* (L) Moench] is a C<sub>4</sub> crop and uses nitrogen (N), CO<sub>2</sub>, solar radiation and water more efficiently than most C<sub>3</sub> crops

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(Anten et al., 1995; Young and Long, 2000), N nutrient is still one of major factors limiting crop yield. Depending on soil N fertility, farmers apply anywhere between 45 and 224 kg N ha<sup>-1</sup> in sorghum production. Although adequate supply of N to crops is fundamental to optimize crop yields, mismanagement of N, such as excessive N application, can result in contamination of groundwater (Jaynes et al., 2001). Therefore, efficient monitoring of plant N status and appropriate N fertilizer management are essential to balance the factors of increasing cost of N fertilizer, the demand by the crop, and the need to minimize environmental perturbations, especially water quality (Jaynes et al., 2001).

Leaf area (LA) and leaf photosynthetic rates (Pn) are directly associated with plant dry matter (DM) production. Sorghum grain yield is closely related to green LA (Borrell and Douglas, 1997) and leaf Pn (Locke and Hons, 1988; Peng et al., 1991). Although C<sub>4</sub> crops have higher photosynthetic N use efficiencies as compared with C<sub>3</sub> crops (Young and Long, 2000), N supply and plant N status considerably affect sorghum leaf area index (Locke and Hons, 1988), leaf Pn, and canopy radiation use efficiency (Muchow and Sinclair, 1994). Leaf N and chlorophyll (Chl) concentrations are important physiological parameters of detecting crop plant N status. Fertilizer N recommendation is traditionally based on soil N status. However, conventional laboratory methods for quantifying these variables from destructive sampling of plant tissues and soil N content measurements are time consuming and costly.

Remote sensing at leaf to landscape scales of crop physiology as affected by environmental stresses has immense potential for timely crop assessment and management without destructive sampling of plants or extensive soil collection procedures (Afanasyev et al., 2001; Daughtry et al., 2000; Filella et al., 1995). Studies have documented that N status of field crops can be assessed using leaf or canopy spectral reflectance data (Thomas and Gausman, 1977; Chappelle et al., 1992; Blackmer et al., 1994; Osborne et al., 2002). Graeff et al. (2001) investigated the impacts of N, P, Mg, and Fe deficiencies on leaf reflectance under the greenhouse conditions and concluded that reflectance measurements might be used to detect nutrient deficiencies in corn. Under field conditions, significant correlations between corn N status and leaf reflectance changes were obtained when leaf N concentration

was lower than 3.0% of leaf dry weight (Graeff and Claupein, 2003). Nitrogen treatments mainly affected spectral reflectance in both red and near-infrared (NIR) regions with the increase in reflectance in the red region and a decrease in reflectance in the NIR region for N-deficient corn canopy (Walburg et al., 1982). In contrast, Blackmer et al. (1996) reported that reflectances near 550 and 710 nm were better for detecting corn plant N deficiencies compared with reflectances at other wavelengths. In general, N deficiency usually decreases leaf Chl concentration resulting in an increase in leaf reflectance in both green (centered 550 nm) and red edge (700–720 nm) ranges (Daughtry et al., 2000; Zhao et al., 2003). However, a variety of causes of plant stresses may result in increased leaf spectral reflectance due to reduced amounts of Chl (Carter and Knapp, 2001).

Although many studies have found that non-destructive measurements of leaf or canopy reflectance can be used for detecting N-deficient stress in corn (Ma et al., 1996; Blackmer et al., 1996), rice (Wang et al., 1998), and wheat (Voullot et al., 1998), relatively fewer studies described the functional relationships between leaf reflectance values and plant physiological variables, especially leaf N concentration. Leaf spectral reflectance properties are closely related to many morphological, physiological, and biochemical parameters (Peñuelas and Filella, 1998). These parameters are associated with not only growth environments, but also crop species and growth stages. Although grain sorghum growth and yield responses to N nutrient and N fertilizer management have received extensive studies (Plenet and Cruz, 1997), to date, there are no reports on identifying the relationships between plant N status and leaf hyperspectral reflectance properties. We hypothesize that the non-destructive measurements of leaf reflectances at some specific wavelengths may be used to estimate leaf N or Chl contents for monitoring of sorghum N status. In order to test our hypothesis and to develop the appropriate reflectance algorithms, an experiment was conducted under outdoor pot-culture conditions. The objectives of this study were to determine the effects of N deficiency on leaf N and Chl concentrations, leaf Pn, and plant DM accumulation and partitioning and to develop algorithms or quantitative relationships between leaf spectral reflectance or reflectance ratios and leaf Chl or N concentrations for sorghum plant N monitoring.

## 2. Materials and methods

### 2.1. Plant culture

An outdoor pot-culture experiment was conducted in the 2001 growing season at the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, MS, USA (33°28'N, 88°47'W). Seeds of sorghum cultivar, DK 44C, were sown on 2 July 2001 in 12-L white polyvinyl chloride (PVC) pots filled with fine sand. The pots were 0.65 m in height and 0.15 m in diameter with a small hole at bottom to drain excess water. Three hundred and sixty pots were arranged in nine rows with additional one border row on each side and oriented east to west with rows 1-m apart. Seedlings were thinned to one per pot at 7 days after emergence. All pots were irrigated using a computer-controlled drip system with half-strength Hoagland's nutrient solution (Hewitt, 1952) to maintain favorable conditions of water and nutrients.

### 2.2. Treatments

Three treatments include: (1) a control irrigated with half-strength Hoagland's nutrient solution throughout the experiment (100% N); (2) reduced N in the nutrient solution to 20% of the control starting 25 days after sowing (DAS, 20% N); and (3) withheld N from the nutrient solution starting 25 DAS (0% N) until the final harvest (58 DAS). Each treatment had three rows with 120 pots (40 pots in each row and six pots per meter row). All plants in both the 20% N and the 0% N treatments received normal half-strength Hoagland's nutrient solution prior to the N treatments. Three individual tanks were used to provide the respective nutrient solutions upon imposition of the treatments. The nutrient solution was modified by substituting  $\text{CaCl}_2$  for  $\text{Ca}(\text{NO}_3)_2$  to allow for different N concentrations (Reddy et al., 1996).

### 2.3. Measurements

During the treatments, three uppermost, fully expanded leaves were sampled from three plants in each treatment every 3 or 4 days at 1100 h. Leaf spectral reflectance was measured immediately using a portable ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO, USA)

with spectral range from 350 to 2500 nm (1 nm intervals). The optical sensor of the spectroradiometer was mounted in the frame of a supplemental light source (ML 902, Makita Corporation, Aichi, Japan) with a 50-mm distance from target leaf surface. A Spectralon white reference panel was used to optimize the instrument to 100% reflectance at all wavebands prior to leaf reflectance measurements. When measuring leaf reflectance, the individual leaves were placed adaxial side up on top of a black polyurethane background.

After measuring leaf reflectance, five leaf discs (38.5 mm<sup>2</sup> each) were immediately punched from each leaf and placed in a vial with 4 mL of dimethyl sulphoxide for Chl extraction. Three replicate leaves were sampled in each treatment, and the sample vials were incubated at room temperature in dark for 24 h to allow for complete extraction of Chl into the solution. Absorbance of the extract was measured using a Pharmacia UltraSpec Pro UV/VIS spectrophotometer (Pharmacia, Cambridge, England) at 470, 648, and 664 nm to calculate concentrations of Chl a and Chl b (Chappelle et al., 1992). The area of individual leaves was determined using a LI-3100 area meter (LI-COR Inc., Lincoln, NE, USA) after collecting the leaf discs. Leaves were then immediately dried at 70 °C for 72 h, weighed, and ground to determine total N concentrations according to standard micro-Kjeldahl procedures (Nelson and Sommers, 1972). Specific leaf weight was calculated as the ratio of leaf dry weight to LA. Concentrations of leaf Chl (i.e. chlorophyll a + b) were expressed on a LA basis in order to determine the relationships between leaf spectral reflectance and Chl concentrations. Leaf N concentrations were expressed on both dry weight basis (g kg<sup>-1</sup>) and LA basis (g m<sup>-2</sup>).

During plant growth, the Pn, stomatal conductance ( $g_s$ ), and intercellular CO<sub>2</sub> concentration ( $C_i$ ) of the uppermost, fully expanded leaves from six plants in each treatment were measured between 1000 and 1200 h using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) at 31, 43, 49, and 58 DAS. When measuring Pn,  $g_s$  and  $C_i$ , the photosynthetically active radiation (PAR), provided by a 6400-02 LED light source, was set to 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , temperature inside the leaf cuvette was set to 30 °C, relative humidity was adjusted to near ambient level, and leaf chamber CO<sub>2</sub> concentration was set to 360  $\mu\text{L L}^{-1}$ . Additionally, photosynthetic light- and  $C_i$ -response curves of the uppermost, fully expanded leaves were

determined from three plants in each treatment between 46 and 50 DAS. When measuring photosynthetic light-response curves, the temperature inside the leaf cuvette was set to 30 °C, and CO<sub>2</sub> concentration in the leaf cuvette was set to 360 μL L<sup>-1</sup>. The PAR was gradually increased from 0 to 2000 μmol m<sup>-2</sup> s<sup>-1</sup> in 11 steps. Leaves were adapted in dark for 20 min prior to logging the first measurement. Values of leaf dark respiration rates ( $R_d$ ) and light compensation points (LCP) were obtained by linear regression of the first four light intensities (PAR: 0, 100, 200 and 400 μmol m<sup>-2</sup> s<sup>-1</sup>) and gas exchange rates. When measuring photosynthetic  $C_i$ -response curves, temperature in the leaf cuvette was maintained at 30 °C and PAR was set to 1500 μmol m<sup>-2</sup> s<sup>-1</sup>. The CO<sub>2</sub> concentration in the cuvette was changed by 10 steps between 0 and 800 μL L<sup>-1</sup>.

Plants were harvested at 58 DAS and separated into leaves, stems and roots. Leaf area was recorded using the LI-3100 leaf area meter. Plant components were dried at 70 °C and weighed to determine the effects of different N treatments on plant DM accumulation and partitioning.

#### 2.4. Experimental design and data analysis

The experiment was designed in a randomized complete block with three replications. There were 40 pots (plants) in each replication (20 plants for destructive leaf sampling and the other 20 for photosynthesis measurements and final harvest). Linear regression was used to determine leaf  $R_d$  and LCP. Best-fit nonlinear regression was employed to determine relationships between leaf CO<sub>2</sub> exchange rates and PAR or  $C_i$ .

Leaf spectral reflectance data were first averaged over 10-nm intervals to decrease the amount of data

for analysis. The reflectance sensitivity to N deficiency was calculated as the percentage changes in reflectance at each waveband for the N-deficient treatments (i.e. the 20% N and the 0% N) compared to the control. Statistical analysis procedures of ANOVA and Fisher LSD tests at  $P = 0.05$  probability level (SAS Institute, 1997) were employed to distinguish among treatments for growth, DM accumulation, leaf Chl and N concentrations, and leaf reflectance. In order to determine relationships between leaf Chl or N concentrations and leaf reflectance values at different wavelengths, data of leaf reflectance, as well as N and Chl concentrations, were first averaged over replicate leaves and then pooled across treatments and sampling dates ( $n = 21$ ). Coefficients of determination ( $r^2$ ) were calculated and used to evaluate linear relationships of leaf N concentrations with reflectance values at the averaged 10-nm intervals throughout the range of 400–2500 nm. Thereafter, the reflectance values at two wavebands with the greatest  $r^2$  values with leaf N or Chl concentrations used as the numerators and the reflectance values at all other wavebands as denominators to calculate reflectance ratios and the  $r^2$  values of the reflectance ratios with leaf N and Chl were further determined.

Additionally, the first derivatives of leaf reflectances ( $dR/d\lambda$ ) in red edge (685–745 nm) were calculated based on the following equation (Lamb et al., 2002):

$$\frac{dR}{d\lambda} = \frac{R_\lambda - R_{(\lambda-1)}}{\Delta\lambda}$$

where  $R_\lambda - R_{(\lambda-1)}$  is the difference in reflectance measured across a single wavelength increment centered at  $\lambda$  and  $\Delta\lambda$  is the wavelength increment ( $\Delta\lambda = 10$ , in this study).

The reflectance ratios and  $dR/d\lambda$  in red edge, which had the greatest  $r^2$  values with leaf Chl or N

Table 1  
Effects of N deficiency on sorghum plant growth and dry matter accumulation

Treatment	Plant height (m)	Leaf area (m <sup>2</sup> plant <sup>-1</sup> )	Dry weight (g plant <sup>-1</sup> )			
			Leaf	Stem	Root	Total
100% N	0.79 ± 0.04	0.78 ± 0.09	46.8 ± 3.6	83.5 ± 8.0	65.1 ± 6.6	195.4 ± 7.9
20% N	0.77 ± 0.01	0.47 ± 0.07	31.4 ± 4.0	79.2 ± 7.1	54.8 ± 5.7	165.4 ± 8.1
0% N	0.63 ± 0.04	0.25 ± 0.04	19.1 ± 2.6	41.9 ± 8.1	55.1 ± 2.6	116.1 ± 7.8
LSD (0.05)	0.12	0.24	11.9	26.9	NS	27.6

Plants were harvested 58 days after sowing. Data are mean ± S.E. of three replicates. Fifteen plants were harvested in each replicate of each treatment.

concentrations, were selected. Data of leaf Chl and leaf N were plotted against the corresponding reflectance ratios or the  $dR/d\lambda$ , and linear regression analyses were also carried out.

### 3. Results

#### 3.1. Plant growth and dry matter accumulation

At the final harvest (58 DAS), plant height did not differ between the control and the 20% N treatments, but plants grown in the 0% N were 20% shorter than the control plants ( $P < 0.05$ ). Both the 20% N and the 0% N-treated plants had significantly smaller LA and less DM accumulation than the control plants (Table 1). Leaf area and total DM decreased by 40 and 15%, respectively, for the 20% N-treated plants and by 68 and 41%, respectively, for the 0% N-treated plants compared to the control plants. Of plant components, N deficiency had the greatest effect on leaf DM. The 20% N and 0% N treatments resulted in a 33 and 59% less leaf dry weight, respectively, compared to the control.

#### 3.2. Leaf N and chlorophyll concentrations and photosynthesis

During the N-deficient treatment, leaf Chl and DM-based N concentrations changed little across the sampling dates for the 100% N (control) and the 20% N treatments, and did not statistically differ between the two treatments at most measuring dates (Fig. 1A and B). However, starting from 20 days after N was withheld from the nutrient solution, the 0% N-treated plants had significantly lower leaf Chl and leaf DM-based N concentrations than the control plants. Leaf area-based N dynamics during the experiment as affected by the N treatments was similar to that of leaf DM-based N and had relatively smaller changes with plant age (data not shown). Averaged across measuring dates, leaf N concentrations on leaf DM basis of the control, 20% N and 0% N-treated plants were 36.6, 32.0, and 21.8  $\text{g kg}^{-1}$ , respectively; the N concentrations on LA basis were 2.12, 1.83, and 1.30  $\text{g m}^{-2}$ , respectively; and the Chl were 624.9, 527.5, and 374.2  $\text{mg m}^{-2}$  leaf area, respectively.

Leaf Pn slightly decreased with increases in plant age for all the three treatments (Fig. 1C). The 20% N

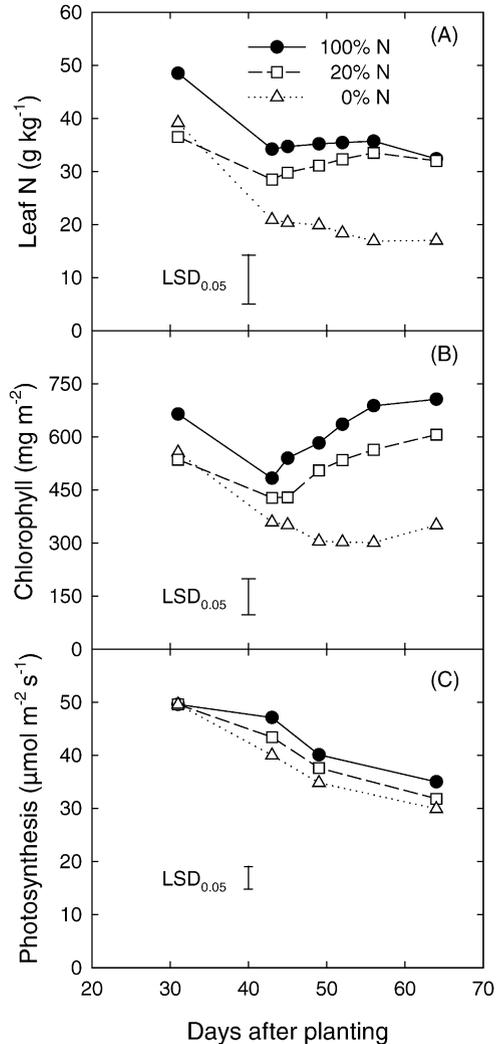


Fig. 1. Changes in sorghum leaf (A) nitrogen and (B) chlorophyll concentrations and (C) net photosynthetic rate during the N-deficient treatments. The LSD values are for means across measurement dates and treatments.

treatment was not statistically different from the control in Pn, but the 0% N treatment had 20% ( $P < 0.05$ ) lower leaf Pn than the control. The N deficiency also decreased leaf  $g_s$ ,  $C_i$ , and  $R_d$  significantly, but did not affect LCP (Table 2). Both leaf Chl concentration ( $r^2 = 0.71^{***}$ ) and leaf Pn ( $r^2 = 0.52^{**}$ ) were linearly correlated with leaf N concentration, but there was no relationship between leaf Chl and Pn (data not shown).

Table 2

Effects of N deficiency on sorghum leaf net photosynthetic rate (Pn), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ), dark respiration ( $R_d$ ), and photosynthetic light compensation point (LCP)

Treatment	Pn ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	$C_i$ ( $\mu\text{L L}^{-1}$ )	$R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	LCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
100% N	42.4 ± 1.9	0.418 ± 0.053	145.2 ± 9.1	4.19 ± 0.59	102.2 ± 9.6
20% N	37.5 ± 2.9	0.310 ± 0.049	104.0 ± 12.4	1.88 ± 0.21	74.7 ± 13.1
0% N	33.9 ± 2.6	0.238 ± 0.035	80.6 ± 10.6	2.24 ± 0.45	75.7 ± 4.6
LSD (0.05)	7.1	0.134	31.1	1.56	NS

The  $R_d$  and LCP are obtained from the photosynthetic light-response curves as shown in Fig. 3. Data are mean ± S.E. [ $n = 12$  (four measuring times × three replicates) for Pn,  $g_s$ , and  $C_i$ ; and  $n = 3$  for  $R_d$  and LCP].

### 3.3. Leaf photosynthetic responses to PAR and $C_i$

Leaf photosynthetic responses to PAR followed similar exponential increases with increases in PAR for all the treatments (Fig. 2A). The differences between the control and the N-deficient plants in leaf Pn increased as PAR increased. When PAR was between 600 and 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , the 20% N-treated plants had a 16–35% lower leaf Pn and the 0% N-treated plants had a 31–41% lower leaf Pn compared to the control plants. The N-deficient plants also had significantly lower  $g_s$  and  $C_i$  (data not shown). No statistically significant difference was detected in photosynthetic light-response curves between the 0% N and the 20% N treatments.

Leaf Pn rapidly increased with increases in  $C_i$  and almost reached the maximum values when  $C_i$  was around 250  $\mu\text{L L}^{-1}$  for all the treatments (Fig. 2B). The differences in photosynthetic  $C_i$ -response curves among the treatments were much smaller compared to those in the photosynthetic light-response curves. Between 50 and 600  $\mu\text{L L}^{-1}$  of  $C_i$ , leaf Pn of the 20% N and the 0% N treatments were only 7 and 14%, respectively, lower than the control.

### 3.4. Leaf hyperspectral reflectance

Although the leaf reflectance spectra showed similar patterns for all the treatments (Fig. 3A), the N deficiency mainly increased the leaf reflectance at two ranges of green (centered 555 nm) and red edge (centered 715 nm) and the reflectances at these two wavebands were the most sensitive to N supply (Fig. 3B). One week after N was withheld from the nutrient solution, leaf reflectance values at 555 and 715 nm for the 0% N treatment increased by 12% and further increased

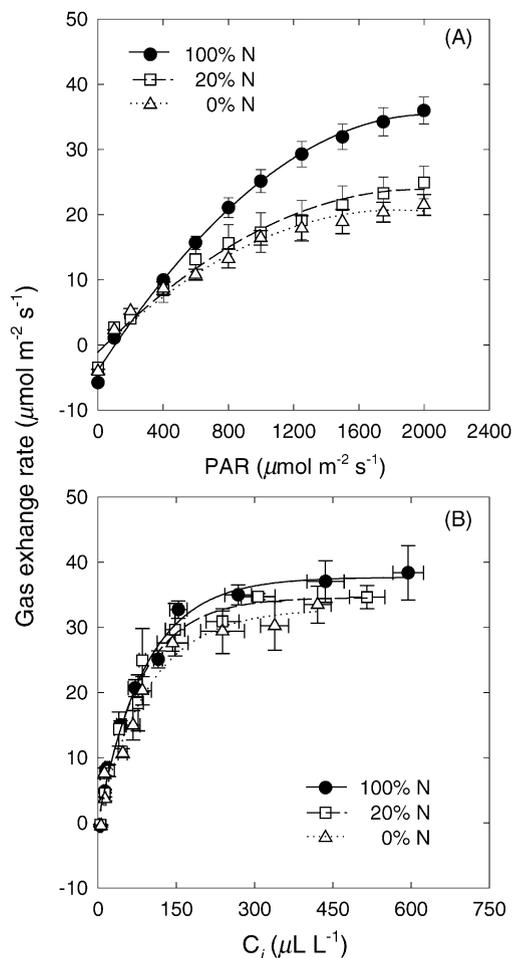


Fig. 2. Leaf photosynthetic responses of sorghum plants to (A) photosynthetically active radiation (PAR) and (B) intercellular CO<sub>2</sub> concentration ( $C_i$ ) as affected by the nitrogen deficiencies. Measurements were taken 46 and 50 days after sowing. Each data point is the mean ± S.E. of three measurements.

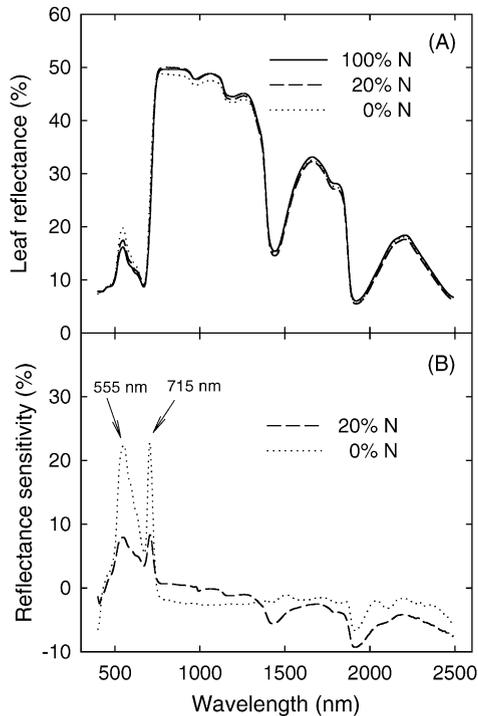


Fig. 3. Effect of nitrogen deficiency on sorghum leaf reflectance properties: (A) leaf spectral reflectance and (B) reflectance sensitivity to N deficiency. Data are means of seven sampling dates and three leaves at each date from 31 to 58 days after sowing. The reflectance sensitivity =  $[(R_{\lambda}$  of N-deficient treatment  $- R_{\lambda}$  of the control)/ $R_{\lambda}$  of the control  $\times 100\%]$  ( $\lambda = 405, 415, 425, \dots, 2495$ ).

by 43% at 4 weeks after the treatment as compared with the control (data not shown).

Simple correlation and linear regression analyses indicated that leaf reflectance values at three regions centered at 405, 715, and 555 or 1075 nm were most closely correlated with leaf N and Chl concentrations among the reflectance values at all 210 wavebands calculated (Fig. 4A). However, reflectances at these single bands could only explain 13–22% of Chl variation and 31–40% of leaf N variation (i.e.  $r^2$  values were only 0.13–0.22 for Chl and 0.31–0.40 for leaf N concentration). Based on the wavebands where leaf reflectances had greatest  $r^2$  values with leaf N and Chl concentrations (Fig. 4A), the reflectance values at the four specific wavebands (405, 555, 715, and 1075 nm) were selected as numerators to calculate two-band reflectance ratios with reflectance values at each of all other wavebands ( $R_{\lambda}$ ) from 400 to 2500 nm. The  $r^2$

values of all the simple reflectance ratios with leaf Chl or N were determined (Fig. 4B–E). The reflectance ratios with the greatest  $r^2$  values with Chl and N concentrations are shown in Fig. 4B–E. Of these reflectance ratios,  $R_{1075}/R_{735}$  and  $R_{405}/R_{715}$  were most closely and linearly correlated with leaf Chl ( $r^2 = 0.66$ ) and leaf DM-based N ( $r^2 = 0.68$ ), respectively. When leaf N concentration was expressed on a LA basis, the best reflectance ratio ( $R_{1075}/R_{735}$ ,  $r^2 = 0.73$ ) for estimating leaf N ( $\text{g m}^{-2}$ ) exactly matched the best reflectance ratio for estimating leaf Chl concentration in Fig. 4E.

Nitrogen deficiency caused red-edge reflectances (Fig. 5A) and the peak of first derivative of reflectances ( $dR/d\lambda$ ) in red edge (Fig. 5B) to shift towards shorter wavelengths (i.e. left side). The  $r^2$  values of the  $dR/d\lambda$  in red edge with leaf Chl and leaf N concentrations are presented in Fig. 5C. Compared to leaf red-edge reflectances at individual wavelengths (see Fig. 4A) and the reflectance ratios selected (Fig. 4C–E), the  $dR/d\lambda$  centered 730 or 740 nm further improved the relationships between leaf reflectances and leaf Chl ( $r^2 = 0.83$ ) or leaf N ( $r^2 = 0.73$  for leaf DW-based N and 0.81 for LA-based N). These selected reflectance ratios ( $R_{1075}/R_{735}$  and  $R_{405}/R_{715}$ ) and  $dR/d\lambda$  in red edge centered 740 or 730 nm increased linearly with increases in leaf Chl and N concentrations (Fig. 6).

## 4. Discussion

### 4.1. Plant growth and physiological responses to nitrogen deficiency

Although sorghum plants use N more efficiently than most  $C_3$ -type crops and are more tolerant to drought and high temperature stresses compared to corn (Young and Long, 2000), N deficiency suppressed plant growth and DM accumulation and allocation (Table 1). Decreased plant biomass production due to N shortage was associated with reductions in both LA and leaf photosynthetic capacity (Sinclair, 1990) and was mainly attributed to a smaller LA in sorghum in the present study (see Fig. 1 and Table 1).

Leaf Chl concentration and Pn were closely correlated with leaf N levels and decreased linearly as leaf N concentration decreased. These results agree with earlier reports in sorghum (Muchow and Sinclair, 1994) and in corn (Wolfe et al., 1988; Muchow and Sinclair,

1994; Settimi and Maranville, 1998; Zhao et al., 2003). Although both leaf Chl concentration and Pn declined linearly as leaf N decreased, the Pn declined less compared to Chl. For instance, when leaf N declined from 50 to 20 mg kg<sup>-1</sup>, leaf Chl decreased by 51%, while

leaf Pn decreased by 33%. The N-deficient plants had a 12% (for the 20% N treatment) or 20% (for the 0% N treatment) lower LA-based Pn than the control plants (Fig. 1 and Table 1), but the former had 12–38% higher Pn per unit Chl when leaf photosynthesis was expressed

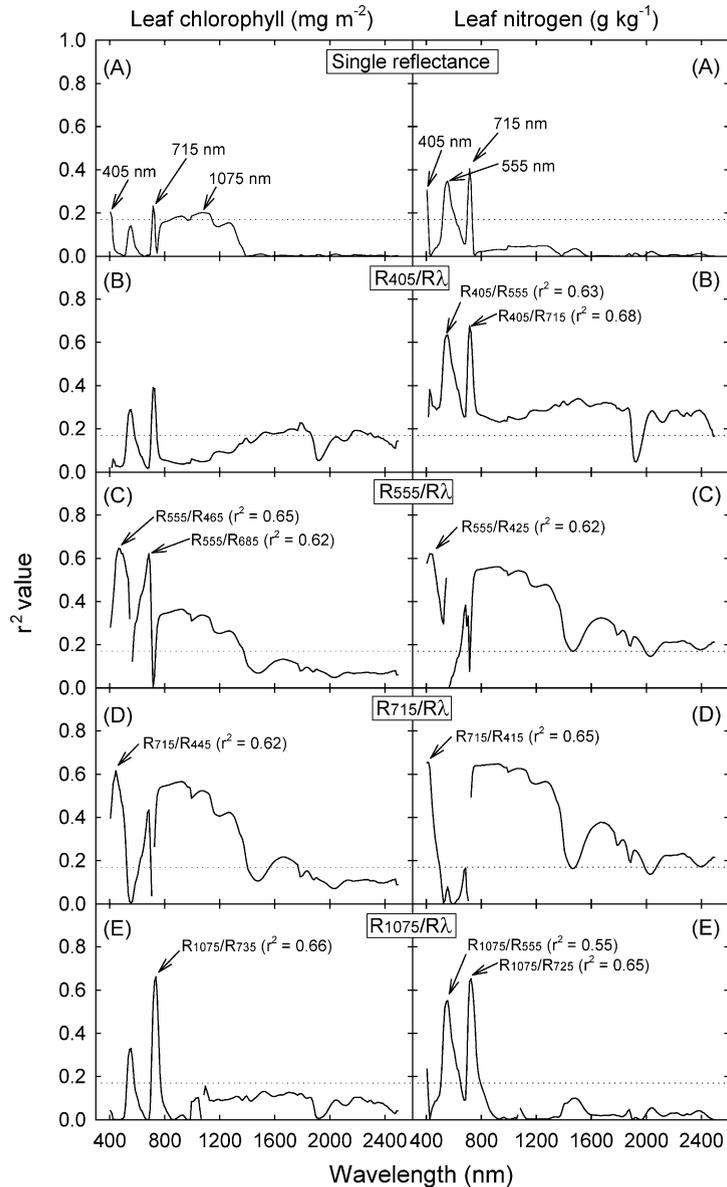


Fig. 4. Coefficients of determinations ( $r^2$ ) vs. wavelengths for the relationships between sorghum leaf chlorophyll (left) or leaf N (right) concentrations and (A) leaf reflectance at all wavelengths from 400 to 2500 nm or reflectance ratios of (B)  $R_{405}/R_\lambda$ , (C)  $R_{555}/R_\lambda$ , (D)  $R_{715}/R_\lambda$ , and (E)  $R_{1075}/R_\lambda$ . The  $r^2$  values were based on a linear model and data were pooled over the three treatments and seven sampling dates ( $n = 21$ ). Dotted lines in the figure represent significant level of  $r^2$  values ( $r^2 = 0.17$  at  $P = 0.05$ ,  $n = 21$ ).

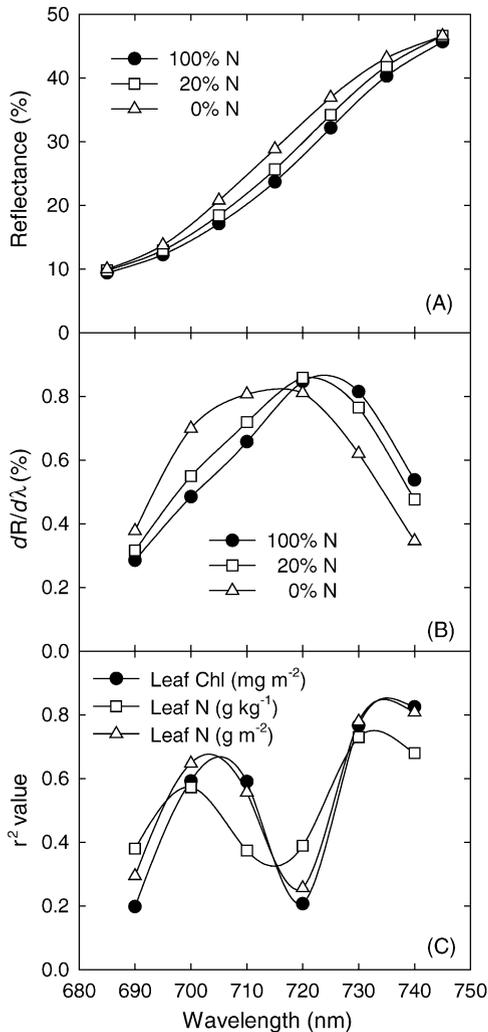


Fig. 5. Effects of nitrogen deficiency on sorghum (A) leaf reflectance in red edge ( $\lambda = 685\text{--}745$  nm) and (B) the first derivative of reflectance ( $dR/d\lambda$ ) in red edge and (C) coefficients of determinations ( $r^2$ ) vs. wavelengths for relationships between leaf Chl or leaf N concentrations and the  $dR/d\lambda$  in red edge.

on Chl basis (data not shown). Our results are in agreement with Robinson and Burkey (1997), who found that the photosystems and photosynthetic electron transport chain components are more active per unit chlorophyll in leaf chloroplast thylakoids of N-limited soybean plants than in thylakoids of N-sufficient plants resulting in a higher leaf  $\text{CO}_2$  assimilation rate per unit chlorophyll of N-limited plants. Although this kind of chlorophyll adjustment may partially mitigate negative

effects of N deficiency on leaf photosynthetic capacity, decreases in both LA-based Pn and LA due to N deficiency are major causes limiting plant growth and productivity (Novoa and Loomis, 1981; Sinclair, 1990; Muchow and Sinclair, 1994; Zhao et al., 2003).

Leaf photosynthesis depends on many physiological and biochemical processes, such as  $g_s$ ,  $C_i$ , photochemical capacity of PSII, and levels and activities of carbon-fixation enzymes. Earlier studies in several other crops indicated that N deficiency reduced either ribulose biphosphate carboxylase/oxygenase (Rubisco) activity (Heitholt et al., 1991) or the amount of the enzyme (Osaki et al., 1993). Recently, Maranville and Madhavan (2002) documented that N deficiency reduced levels of both phosphoenolpyruvate carboxylase (PEPcase) and Rubisco in sorghum leaves. Ciompi et al. (1996) reported that under N-deficient stress, the decline in sunflower (a  $C_3$  crop) leaf photosynthesis and the rise in  $g_s$  were accompanied by an increase in  $C_i$ . Therefore, the decline in leaf photosynthetic activity of N-deficient sunflower at light saturation level was due to a reduced mesophyll activity, rather than stomatal limitation. Similar conclusions were drawn in cassava crop by Cruz et al. (2003). In contrast, our results indicate that under N deficiency, decreased  $g_s$ , resulting in lower  $C_i$ , seemed to be the major cause of limiting sorghum leaf Pn because both  $g_s$  and  $C_i$  decreased simultaneously with the decrease in leaf Pn (Table 2). At the same  $C_i$ , the differences in the leaf Pn of all the three N treatments were much smaller (Fig. 2B), compared to their photosynthetic light-response curves (see Fig. 2A). Therefore, the decrease in stomatal conductance seems to be the first cause of the reduction in sorghum leaf Pn under N deficient conditions. Thus, N deficiency in sorghum affected not only leaf Pn but also leaf transpiration and water use efficiency.

#### 4.2. Relationships between leaf spectral reflectance and leaf chlorophyll or N

The results of N deficiency increasing sorghum leaf reflectance in two narrow ranges centered at 555 and 715 ( $\pm 5$ ) nm in the present study are consistent with several earlier reports in buffelgrass (Everitt et al., 1985), corn (Blackmer et al., 1994, 1996; Zhao et al. 2003), and cotton (Zhao et al., 2005). Therefore, the reflectances centered at 555 and 715 nm may be used to detect plant N deficiencies of most field crops.

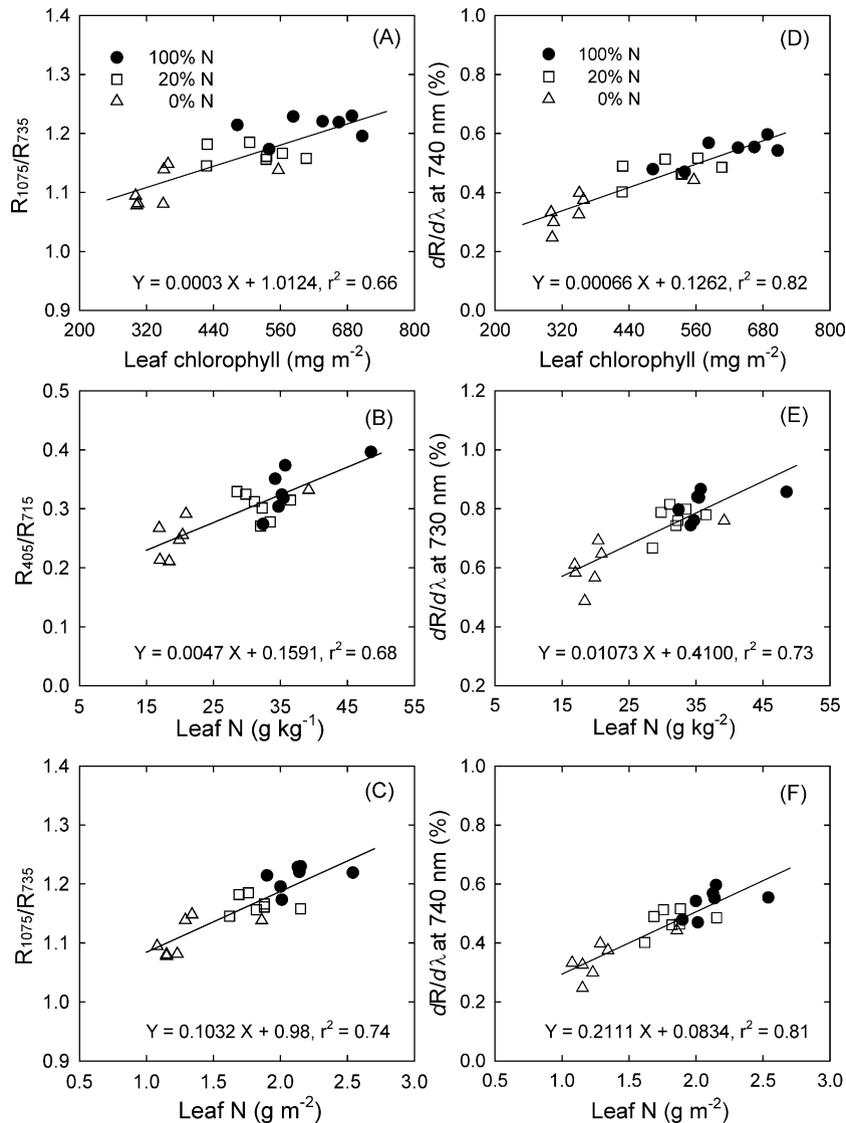


Fig. 6. Linear regression of sorghum (A and D) leaf chlorophyll, (B and E) leaf dry weight-based N, or (C and F) leaf area-based N concentrations with (A–C) a given reflectance ratio or with (D–F) the first derivative of reflectance ( $dR/d\lambda$ ) at 730 or 740 nm, which has the greatest  $r^2$  value with leaf chlorophyll or N concentrations. Data are pooled over the three treatments and seven sampling dates from 31 to 58 days after sowing ( $n = 21$ ).

Blackmer et al. (1996) found that reflectances near 550 and 710 nm were superior to reflectance near 450 and 650 nm for detecting corn N deficiencies. In fact, the effects of N deficiency on leaf reflectance at these wavebands were attributed to changes in leaf Chl levels (Carter and Knapp, 2001). Leaf Chl and N concentrations are important indicators of crop plant N diagnosis. Filella et al. (1995) reported that wheat canopy

reflectance values at 550 and 680 nm, and the red-edge regions were significantly correlated with canopy Chl a content.

Although sorghum leaf reflectance at 555 and 715 nm was most sensitive to N supply in our study, the linear relationships between the reflectance values at these two wavebands and leaf Chl and N were very low (see Fig. 4A). When reflectance ratio data sets were

created based on reflectance values at the two most N-sensitive bands (555 and 715 nm) and other two wavebands (405 and 1075 nm) with the greatest  $r^2$  values with leaf Chl or leaf N, however, leaf Chl and N concentrations were most highly correlated with  $R_{1075}/R_{735}$  and  $R_{405}/R_{715}$ , respectively (see Fig. 4). Compared with the reflectance at single wavebands, the reflectance ratios significantly improved precision of estimating leaf Chl and N concentrations in sorghum. In addition, several other reflectance ratios may also be used to estimate sorghum leaf Chl and N concentrations. These ratios include  $R_{555}/R_{465}$ ,  $R_{555}/R_{685}$  and  $R_{715}/R_{445}$  for Chl and  $R_{405}/R_{555}$ ,  $R_{555}/R_{425}$ ,  $R_{715}/R_{415}$ ,  $R_{1075}/R_{555}$ , and  $R_{1075}/R_{725}$  for leaf DM-based N. Several other studies also reported that leaf reflectance ratios were superior to single reflectance values in predicting leaf Chl or N status in different crops in spite of inconsistency in the wavebands used in calculating reflectance ratios (Tarpley et al., 2000; Carter and Spiering, 2002; Read et al., 2002; Zhao et al., 2003). Although the N-sensitive wavebands (i.e. centered 555 and 715 nm) are common for most crops, the functional algorithms of expressing relationship between leaf N or Chl concentration and reflectance or reflectance ratios appears to be species and experiment dependent.

A red-edge shift to shorter wavelengths with reduced N supply in the present study is consistent with earlier studies in ryegrass (Lamb et al., 2002) and in cotton (Fridgen and Varco, 2004). When regressing leaf Chl and leaf N concentrations with the first derivatives of leaf reflectance ( $dR/d\lambda$ ) in red-edge region, the linear relationships between leaf Chl or leaf N and the  $dR/d\lambda$  centered 740 or 730 nm were further improved ( $r^2 = 0.73$ – $0.82^{***}$ , Fig. 6D–F) compared to the reflectance ratios ( $r^2 = 0.66$ – $0.74$ , Fig. 6A–C). Therefore, sorghum leaf Chl and N concentrations could be more precisely estimated using either the  $dR/d\lambda$  at the red edge or the particular reflectance ratios developed in this study.

## 5. Conclusions

Nitrogen deficiency decreased LA, Chl content, and Pn of sorghum plants, resulting in lower DM accumulation. Decreased sorghum leaf photosynthetic capacity due to N deficiency was mainly associated with the decreases in  $g_s$  and  $C_i$  rather than mesophyll activity.

Leaf spectral reflectance, especially in visible and red-edge regions, was very sensitive to sorghum plant N status. Nitrogen deficiency mainly increased leaf reflectance at 555 and 715 ( $\pm 5$ ) nm. Changes in leaf reflectance at these spectral regions were mainly related to either N or Chl level of leaves. Leaf Chl and N concentrations were highly and linearly correlated not only with the  $dR/d\lambda$  at 730 or 740 nm, but also with the reflectance ratios of  $R_{1075}/R_{735}$  and  $R_{405}/R_{715}$  with the greatest  $r^2$  values. These specific  $dR/d\lambda$  or reflectance ratios could be used for estimating sorghum leaf Chl and N concentrations. Therefore, nondestructive measurements of leaf spectral reflectance at these narrow wavebands may provide a rapid and inexpensive tool for in-season estimation of leaf Chl and N contents and for site-specific N management in sorghum production.

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