


Portable Chlorophyll Meter for Indirect Evaluation of Photosynthetic Pigments and Nitrogen Content in Sweet Sorghum

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Abstract Portable chlorophyll meters can be a useful tool for nitrogen (N) determination in crops, particularly in those that show a high correlation between N levels in plants tissues and chlorophyll readings. However, there is a lack of information regarding these correlations in some crops, such as sweet sorghum. The aim of this study was to determine the relationship between the photosynthetic pigments extracted in dimethylsulfoxide and N content in leaves with the readings of a portable chlorophyll meter (ClorofiLog[®]), as a function of the application of N doses in sweet sorghum. The experimental design was a split-plot, with five N doses applied at sowing (0, 15, 30, 45 and 60 kg N ha⁻¹) in the main plot and as subplots were considered five topdressing of N doses in order to reach 0, 100, 200, 300 and 400 kg N ha⁻¹. At 25 and 43 days after emergence, portable chlorophyll meter readings, destructive determinations of photosynthetic pigments and N content in sorghum leaves were measured. At harvesting, biometric and yield characteristics of plants were determined. ClorofiLog[®] was not able to estimate N status at V5 stage, but it can assess the N status in sorghum leaves at V9 stage. At the latter stage, topdressing N promoted greater increases on dry mass, the chlorophyll *b* index (obtained via ClorofiLog[®]) and total chlorophyll (obtained by the destructive method).

Keywords ClorofiLog[®] · Photosynthesis · Malibu 5010 · Nitrogen fertilization · *Sorghum bicolor*

Abbreviations

N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
CEC	Cation exchange capacity
pH	Hydrogen potential
OM	Organic matter
V	Base saturation
H	Hydrogen
Al	Aluminum
SB	Sum of bases
Cl _a	Clay
San	Sand
Si	Silt

Introduction

Ethanol production in Brazil depends on sugar cane cultivation, and it is important to identify alternative crops that can be grown in the inter-season (Castro et al. 2016). Sweet sorghum (*Sorghum bicolor* L. Moench) can be an interesting alternative of raw material for ethanol production (Rooney et al. 2007), since it has succulent stems, it is rich in glucose, sucrose and fructose, and it has a °Brix ranging from 14 up to 22%. Thus, the resulting broth can be fermented to ethanol. Besides, sweet sorghum can be grown in the late spring and early summer (off-season of

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sugarcane), ensuring the supply of raw material to the industries during a longer period of time (Wu et al. 2010).

In order to increase sorghum cultivation in Brazil, it is important to fulfill some gaps related with its management. Regarding nutrient management, nutrition-related studies are still scarce, and recommendations for corn fertilization are often used (Santos et al. 2015).

It's well-known that N is one of the nutrients that has high potential to reduce sugar yield. In sweet sorghum, specific studies are recent, and the results are diverse. Sawargaonkar et al. (2013) reported that high N doses may increase vegetative growth and reduce sugar concentration in the stems. On the other hand, some results show a linear increase in sweet sorghum yield increasing N doses up to 200 kg N ha⁻¹ (Santos et al. 2015). Other results highlighted the low N demand by the crop (Barbanti et al. 2006).

In modern agriculture, the use of sensors, such as portable chlorophyll meters, is one of the most studied techniques in order to increase N use efficiency. In addition to being a constituent of the proteins that participate in carbohydrate metabolism, almost 70% of the N in plants is incorporated in the chloroplast-related enzymes that carry out photosynthesis. Thus, there is an interdependence between N compounds and chlorophyll (Singh et al. 2012).

Some researchers have demonstrated the existence of a relationship between the index of chlorophyll and the N status in the leaves of several species, such as citrus (Jifon et al. 2005), coffee (Torres Netto et al. 2005) and maize (Amarante et al. 2010; Kalaji et al. 2017), wheat, rice and oats (Argenta et al. 2001). A good association between the chlorophyll meter SPAD-502 index and the chlorophyll content (obtained by the destructive method) in plants has been reported in some crops, such as sugarcane (Jangpromma et al. 2010), soybean (Rodriguez-Lucena et al. 2010) and common bean (Silveira et al. 2003).

Thus, it is possible to estimate leaf N content and select the adequate N doses using portable devices such as ClorofiLog[®]. However, for sweet sorghum there is a lack of information regarding the effectiveness of this tool. This device uses photodiodes emitting at three wavelengths: two emit within the red band, close to the peaks of each type of chlorophyll ($\lambda = 635$ and 660 nm) and one in the near infrared ($\lambda = 880$ nm). In this way, an inferior sensor receives the radiation transmitted through the leaf structure, and using this data the device provides the relative indexes of chlorophyll (Falker 2008).

After the proper calibration of the chlorophyll meter, it is possible to estimate the N status in the plants and use the information to make decisions about when and how much N to apply. In this context, the aim of this study was to determine the relationship between the photosynthetic pigments extracted in DMSO and N content of leaves with

the readings obtained from the portable chlorophyll meter (ClorofiLog[®]), as a function of the application of N doses in sweet sorghum.

Materials and Methods

Experimental Area

The experiment was carried out in the experimental area of Nexsteppe Sementes do Brasil, in Rio Verde, GO, Brazil (17° 47' 50" S, 50° 54' 0" W and altitude of 730 m). According to the Köppen classification, the regional climate is a Tropical Savanna (AW) with dry winter and rainy summer. Before planting, soil sampling was performed at 0.0–0.2 and 0.2–0.4 m depth to assess chemical attributes and clay content (Table 1) and the soil was classified as Rhodic Hapludox (USDA-Soil Survey Staff 2010).

Experimental Design and Procedure

The experimental design was a randomized block with a split-plot scheme and four replicates. The treatments allocated in the main plots were N doses applied at sowing (0, 15, 30, 45 and 60 kg N ha⁻¹), and topdressing N fertilization were considered as subplots (to reach the final doses of 0, 100, 200, 300 and 400 kg N ha⁻¹).

Each plot had an area of 62.5 m² divided in five subplots of 12.5 m², with six plant rows spaced 0.5 m apart. The hybrid Malibu 5010 from Nexsteppe was sown in rows, using a seed density of 20 kg ha⁻¹ to reach a plant population density of 120,000 plants ha⁻¹. Urea (45% of N) was used as N source, and it was applied as topdressing (manually and without incorporation) at 26 days after emergence (DAE). The rainfall in the experimental area and the temperature variation during the period of conduction of study are presented in Fig. 1 (Inmet 2014).

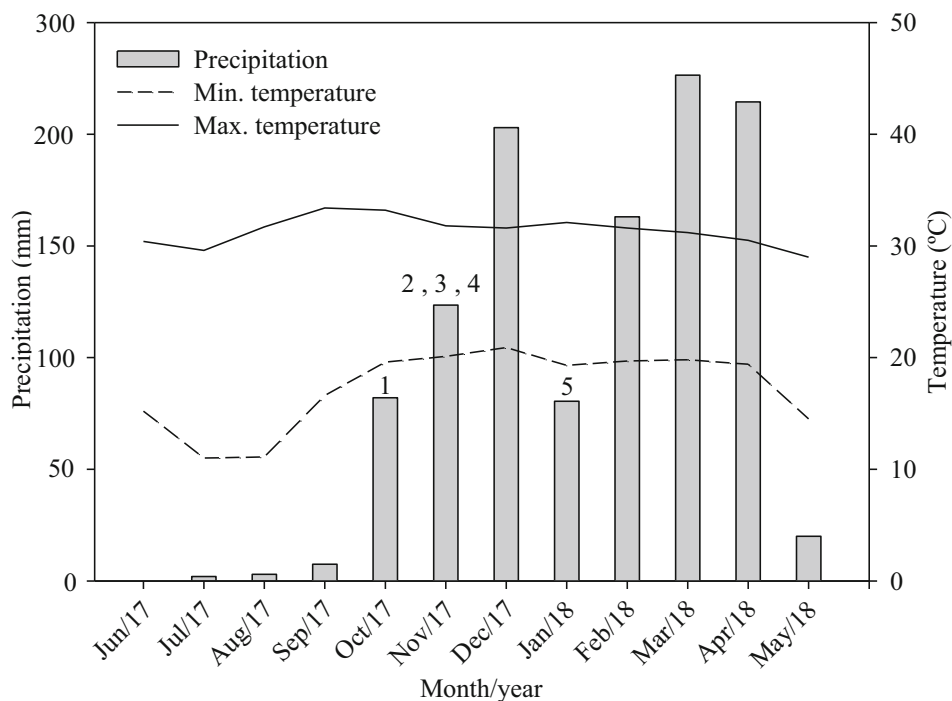
Measurements

Sensors readings were taken at 25 DAE (before topdressing fertilization) and 43 DAE, when sorghum plants were, respectively, at V5 and V9 stage. The portable chlorophyll meter ClorofiLog[®] model, Falker, Brazil, was used generating readings of chlorophyll *a*, chlorophyll *b* and total chlorophyll. In each plot, three plants were chosen and two readings were performed in each plant, in the middle third of the last fully expanded leaf, which defines each stage of development.

In both plant stages (V5 and V9), after chlorophyll meter readings, leaf disks were collected—in the same place where readings were taken—using a cast metal perforator with an area of 115 mm². The leaf disks were put in

Table 1 Chemical and textural characteristics of the soil of the experimental area

Depth m	pH CaCl ₂	OM g dm ⁻³	P mg dm ⁻³	K cmol _c dm ³	Ca dm ³	Mg	H + Al	SB	CEC	V %	Cl _a	San	Si
0–0.2	5.60	40.6	14.1	0.3	2.6	1.2	3.1	4.1	7.0	56	45	34	21
0.2–0.4	4.98	31.4	12.6	0.1	1.5	0.5	4.1	2.1	6.2	36	48	37	15

Fig. 1 Rainfall (mm) and monthly temperature average observed in the experimental area in Rio Verde, GO, Brazil

1 - N doses applied at sowing. 2 - Chlorophyll meter readings (at stage V5).
 3 - N doses applied at coverage (at stage V5). 4 - Chlorophyll meter readings (at stage V9).
 5 - Harvest

test tubes, individually wrapped in aluminum foil to protect against solar radiation and placed in a dry icebox in order to prevent the denaturation of enzymes. Then, five plants per plot were sampled and separated in stem and leaves, to dry mass determination at 70 °C for 48 h, and five completely expanded leaves were taken to N determination, according to Malavolta et al. (1997).

Chlorophyll *a*, *b* and total were determined following methodology described by Hiscox and Israelstam (1979), after disk samples dissolution using 5 mL of dimethylsulfoxide reagent (DMSO) and incubation at 70° C for 30 min in a water bath, the samples were extracted and determined by spectrophotometry.

Harvest was performed at 105 DAE, on two central lines of each plot, totaling 6 m². Stem diameter and number of tillers also were measured. Plants fresh mass was weighed

and then oven-dried at 70 °C for 48 h to estimate dry weights.

Nitrogen accumulation, stem moisture, broth °Brix and estimated ethanol productivity were also determined.

Data Analysis

Data obtained at V5 stage were analyzed using analysis of variance (ANOVA). Regression analyses were used to describe N doses effect in chlorophyll content and dry mass. The parameters used to choose the best polynomial regression model were the *F*-test significance ($P < 0.05$), predicted and adjusted R^2 (higher than 0.8), and the residual plots independence (including Durbin–Watson test to verify correlation between adjacent residuals). If more than one model met these criteria, the model with the lower number of parameters was chosen.

Data of V9 stage were subjected to ANOVA as a split-plot design with basal N doses in the main plots and N topdressing doses in the subplots. When there was no interaction effect, data were analyzed using regression analyses, as described before. However, when interactions were significant using the *F*-test, they were partitioned using a response surface methodology and the isolated effect of each factor was disregarded.

Pearson correlation (*R*) among chlorophyll *a*, *b* or total chlorophyll and readings of ClorofiLog[®] meter and total N were also evaluated using the Sisvar software (Ferreira 2014).

Results and Discussion

Parameters Evaluated After Sowing Fertilization (V5)

Relationship Between Chlorophylls Index (Destructive Method and Nondestructive) and Effects of N Doses in the Plant

At 25 DAE, there was no correlation between N doses, N levels in plant tissues, dry mass and *a*, *b* and total chlorophyll content, determined by both ClorofiLog[®] readings and destructive method (Table 2).

Chlorophyll levels estimated using ClorofiLog[®] can differ from those obtained by destructive methods, since chlorophyll distribution in leaves can be uneven, mainly in leaves with high chlorophyll content, resulting in underestimation of chlorophyll by ClorofiLog[®] (Uddling et al. 2007).

Increasing N levels in soil can result in higher chlorophyll content in plants, since N is a structural component of chlorophyll molecules. However, usually it performs a nonlinear correlation, reaching a plateau, and after that increasing N content in plant tissues is not followed by chlorophyll content increases (Argenta et al. 2001). Soils with high OM, as observed in this study (Table 1), usually

has high N content, so this plateau can be reached and N application may not increase chlorophyll content, mainly in the early stages of crop development, when soil N may meet N needs of the crop. In fact, sweet sorghum has a low N demand in the early developmental stages (Soares et al. 2014). Additionally, after N uptake by plant, N conversion into chlorophyll takes some time. Considering all these facts, N determination by ClorofiLog[®] may are not precisely up to V5.

Biomass and Chlorophyll Content in Response to N Fertilization

Nitrogen application at sowing had no effect on total dry mass accumulation and chlorophyll *a*, *b* and total chlorophyll estimated by ClorofiLog[®] at V5 stage, which average of 6.75 g per plant, 31.5, 6.6 and 38.1, respectively (Table 3).

Chlorophyll measured by the destructive method had a slightly difference among treatments for chlorophyll *a*, *b* and total chlorophyll values. The highest values of chlorophyll *a* and total chlorophyll were found at the dose of 25 kg N ha⁻¹. Chlorophyll *b* content presented the highest content with application of 20 kg N ha⁻¹ at sowing (Fig. 2a). Nitrogen levels ranged from 39.16 to 41.72 g N kg⁻¹, and thus leaf N content confirms, in part, the luxury consumption by plants (Fig. 2b).

The quadratic adjustment of the equation reveals that the application of 20 kg N ha⁻¹ at sowing was enough to maintain a high N supply, considering the adequate N content in leaves of 32.16 up to 35.33 for different doses and cultivars of sweet sorghum as proposed by Soares et al. (2014).

This high N content in plant tissues can be explained by the high N availability on soil, resulting in higher N uptake by sorghum plants (Table 1), which is accumulated in the plant cell organelles, especially in vacuoles, characterizing the “luxury consumption” (Argenta et al. 2001).

Several papers reported that chlorophyll levels allow the indirectly assessment of the N status in plants (Uddling

Table 2 Pearson correlation (*R*) among chlorophyll *a* (Chlor *a*), *b* (Chlor *b*) and total chlorophyll (Chlor total) determined by ClorofiLog[®] readings and destructive method, N content, dry mass and N doses in sweet sorghum leaves at stage V5

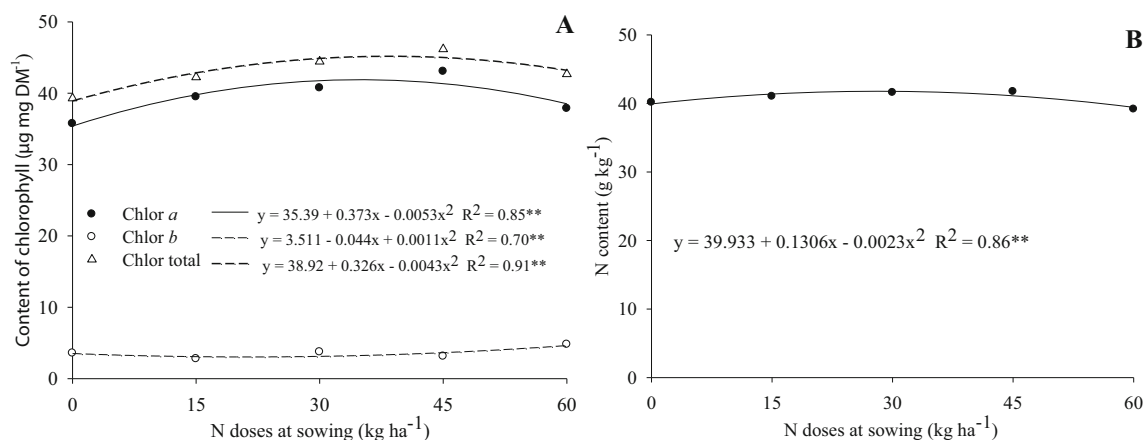
	Chlor <i>a</i>	Chlor <i>b</i>	Chlor total	Dry mass	N content	N doses
N doses	– 0.05 ^{ns}	0.07 ^{ns}	0.01 ^{ns}	0.16 ^{ns}	– 0.11 ^{ns}	–
N content	0.38 ^{ns}	– 0.36 ^{ns}	0.08 ^{ns}	– 0.31 ^{ns}	–	–
Chlor <i>a</i> (DMSO)	0.10 ^{ns}	–	–	0.01 ^{ns}	0.51 ^{ns}	0.40 ^{ns}
Chlor <i>b</i> (DMSO)	–	0.15 ^{ns}	–	0.04 ^{ns}	– 0.39 ^{ns}	0.48 ^{ns}
Chlor total (DMSO)	–	–	– 0.13 ^{ns}	0.02 ^{ns}	0.44 ^{ns}	0.52 ^{ns}

^{ns}Not significant at 0.05 probability

Table 3 Chlorophyll *a* (Chlor *a*), *b* (Chlor *b*) and total (Chlor tot) determined by ClorofiLog[®] reading and total dry mass (TDM) in sweet sorghum at stage V5

N doses applied at sowing kg ha ⁻¹	Chlor <i>a</i> Index ClorofiLog [®]	Chlor <i>b</i>	Chlor tot	TDM g per plant
0	31.5	6.71	38.2	6.25
15	31.7	6.28	38.0	6.75
30	31.6	6.82	38.4	7.00
45	31.6	6.15	37.8	6.25
60	31.3	7.03	38.3	7.50
Means	31.5 ^{ns}	6.60 ^{ns}	38.14 ^{ns}	6.75 ^{ns}

^{ns}Not significant at 0.05 probability

**Fig. 2** Chlorophyll *a*, chlorophyll *b* and total chlorophyll content (a) determined by the destructive method and N (b) content in leaves of sorghum at stage V5 as a function of N doses applied at sowing. **Significant at 0.01 probability

et al. 2007; Coelho et al. 2010; Samborski et al. 2009), since this nutrient is involved in amino acids synthesis, which is associated with the chloroplasts (Rambo et al. 2004). Moreover, N is a structural element in chlorophyll molecules (Singh et al. 2012). Thus, many studies have been carried to develop algorithms that translate the value provided by the portable sensor into decisions about how much N to apply in crops (Ramirez 2010; Solari et al. 2010).

A recent study recommended 20 up to 30 kg N ha⁻¹ at sowing to produce 50 Mg ha⁻¹ of sorghum stems (green mass), in soils with high levels of fertility (Santos et al. 2015). These results support the findings of our study, where it was verified that, sorghum plants presented higher content of chlorophyll *a*, chlorophyll *b*, total chlorophyll measured by the destructive method. These results associated with the ClorofiLog[®] readings may indicate the critical chlorophyll readings meaning the levels above the ones which no response to N fertilizer application is expected.

Parameters Evaluated After Topdressing N Application (V9)

Relationship Between Chlorophylls Index (Destructive Method and Nondestructive) and Effects of N Doses in the Plant

There was a significant correlation among *a*, *b* and total chlorophyll, estimated by ClorofiLog[®] or destructive methods, and dry mass production (DMP), with the coefficient of determination ranging from 0.50 up to 0.89. However, these measurements were not correlated with N doses (Table 4).

Total chlorophyll estimated by ClorofiLog[®] had a positive correlation with DMP and N content, with a coefficient of determination of 0.70 and 0.40, respectively. Moreover, *a*, *b* and total chlorophyll, determined by the destructive method, strongly correlated with DMP (*R* higher than 0.85).

Table 4 Data dispersion and Pearson correlation (*R*) between chlorophyll *a* (Chlor *a*), *b* (Chlor *b*) and total (Chlor total) determined by spectrophotometer reading, ClorofiLog[®], N content, dry mass and N doses in sweet sorghum leaves

	Chlor <i>a</i>	Chlor <i>b</i>	Chlor total	Chlor <i>a</i> (DMSO)	Chlor <i>b</i> (DMSO)	Chlor total (DMSO)	N content
N doses	0.30 ^{ns}	0.46 ^{ns}	0.35 ^{ns}	0.40 ^{ns}	0.48 ^{ns}	0.52 ^{ns}	0.04 ^{ns}
N content	– 0.10 ^{ns}	– 0.18 ^{ns}	0.40*	– 0.14 ^{ns}	– 0.23 ^{ns}	– 0.19 ^{ns}	–
Dry mass	0.50*	0.81**	0.70*	0.85*	0.89**	0.87**	– 0.15 ^{ns}

*Significant at 0.05 probability

**Significant at 0.01 probability

^{ns}Not significant at 0.05 probability

Significant correlations between N content in leaves and chlorophyll meter readings has been reported for beans (Barbosa Filho et al. 2008; Silveira et al. 2003), potato (Coelho et al. 2010), maize (Amarante et al. 2010; Kalaji et al. 2017), wheat (Pias et al. 2014; Cerovic et al. 2005) and other crops (Torres Netto et al. 2005). In leaves, 50 up to 70% of the N are a structural component of chlorophyll molecules or enzymes related to chloroplasts (Singh et al. 2012).

The ClorofiLog[®] showed a directly correlation with extractable chlorophyll, with coefficient of determination of 0.70, 0.90 and 0.81 for *a*, *b* and total chlorophyll, respectively (Fig. 3a–c). Similar results were obtained with soybean (Markwell et al. 1995) and corn (Argenta et al. 2001). However, significant correlation with N status was observed only with total chlorophyll (Fig. 3d).

Anyway, chlorophyll readings provided by portable devices can replace standard N determinations, since these

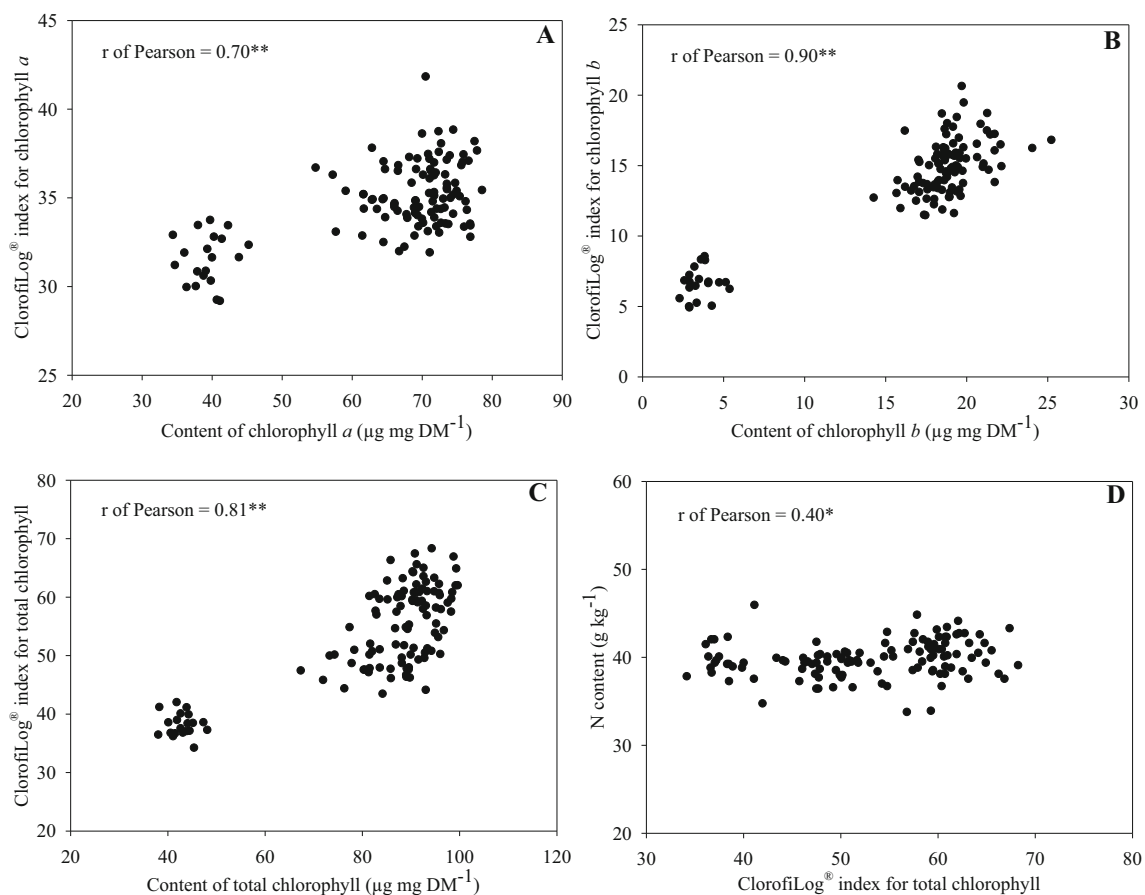


Fig. 3 Data dispersion and Pearson correlation (*r*) between chlorophyll *a* (a), *b* (b) and total chlorophyll (c) determined by spectrophotometer reading, ClorofiLog[®] and N (d) content in sweet sorghum leaves. *Significant at 0.05 probability; **significant at 0.01 probability

measurements have low cost, they are easier to take and it provides a real time N quantification, allowing a higher number of measurements, which is important for variable rates application of fertilizers, and promptly correcting swards N deficiencies through fertilization.

When high N doses are applied, N absorption may not be followed by an increase in chlorophyll content, which is expected since plants will not produce more chlorophyll molecules than they need. In this way, the surplus of N is accumulated as nitrate and cannot be quantified by chlorophyll meter readings (Dwyer et al. 1995), decreasing the coefficient of determination (Blackmer et al. 1995; Argenta et al. 2001; Cerovic et al. 2005).

It is important to emphasize that the models that describe the correlation between readings made with ClorofiLog[®] differ for each culture, due to the intrinsic characteristics of each species, so that each culture requires an independent calibration (Uddling et al. 2007).

The evaluation of photosynthetic pigments by classical methods of detection, based on the use of solvents such as DMSO, presents high diffusive capacity through the membranes of the leaves and are very efficient in the dissolution of the molecules, which facilitates detection (Hiscox and Israelstam 1979). However, they are destructive, costly methods and they not allowed an in situ determination as observed with chlorophyll meter devices.

Biomass and chlorophyll content in response to N fertilization

Increasing N doses did not alter, chlorophyll *a* and total chlorophyll parameters measured by ClorofiLog[®], which presented means of 40.5 and 55.5, respectively. The ClorofiLog[®] for chlorophyll *b* did not show significant interaction among the sources of variation analyzed. However,

it there was a quadratic model adjustment following increasing N doses application at topdressing (Fig. 4a).

The highest increase in chlorophyll *b* was obtained with doses of 200 kg N ha⁻¹, corroborating Pereira Filho et al. (2013), who obtained the greatest increments of fresh mass production and °Brix applying 200 kg N ha⁻¹ in sweet sorghum BR 506 cultivated in soils with high fertility.

Light is a fundamental resource for plant growth and development, because many plant morphological responses depend not only on the presence, attenuation or absence of light, but also on the variation of its spectral quality (Morini and Muleo 2003). It is known that in shaded environments there is a greater transmittance of light in the range of blue (400–500 nm), indicating that the energy balance may favor the energy absorption by chlorophyll *b*, since they are associated with the reaction centers of photosystem II and have the ability to aid in the photosynthetic performance of the plant and attenuate cells photooxidation, eventually resulting in productivity gains. (Nakazono et al. 2001).

Regarding destructive analysis at V9, the levels of chlorophyll *a*, *b* and leaf N content were not affected by increasing N doses applied at sowing and in topdressing. These parameters presented mean values of 69.3 µg mg⁻¹, 19.1 µg mg⁻¹ of dry mass and 39.6 g N kg⁻¹, respectively. The total chlorophyll analysis was significantly adjusted as a function of the N application in topdressing, so that the highest total chlorophyll value was 90.9 µg mg⁻¹ obtained with the application of approximately 200 kg N ha⁻¹ (Fig. 4b).

Regarding the biomass parameters measured in V9, no significant difference was observed in dry mass leaves, which presented a mean of 28.1 g per plant. There was an interaction effect between factors (N fertilization timing) for stem dry mass and total dry mass. The increment of stem dry mass of 29.5 g per plant obtained with the

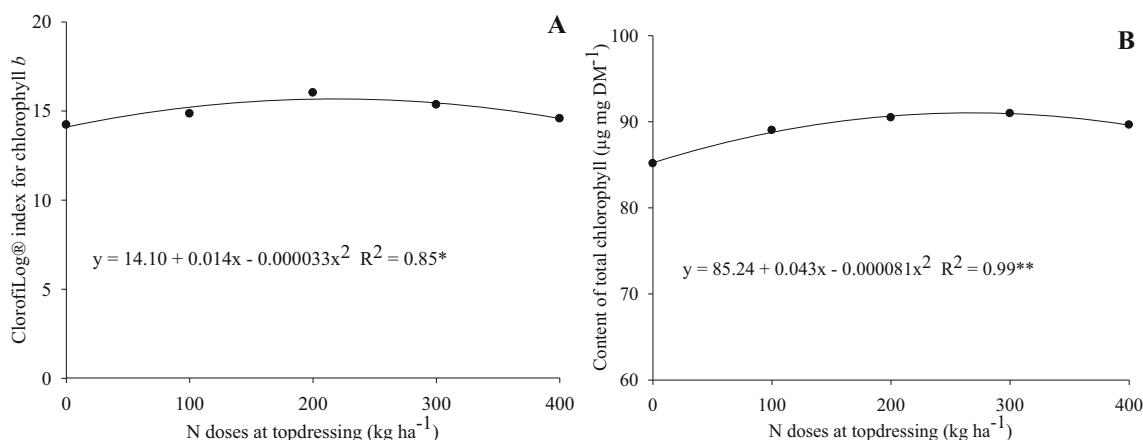


Fig. 4 ClorofiLog[®] for chlorophyll *b* (a) and total chlorophyll content determined by spectrophotometer reading (b) on leaves of sorghum at V9 stage as a function of N doses applied at coverage. *Significant at 0.05 probability; **significant at 0.01 probability

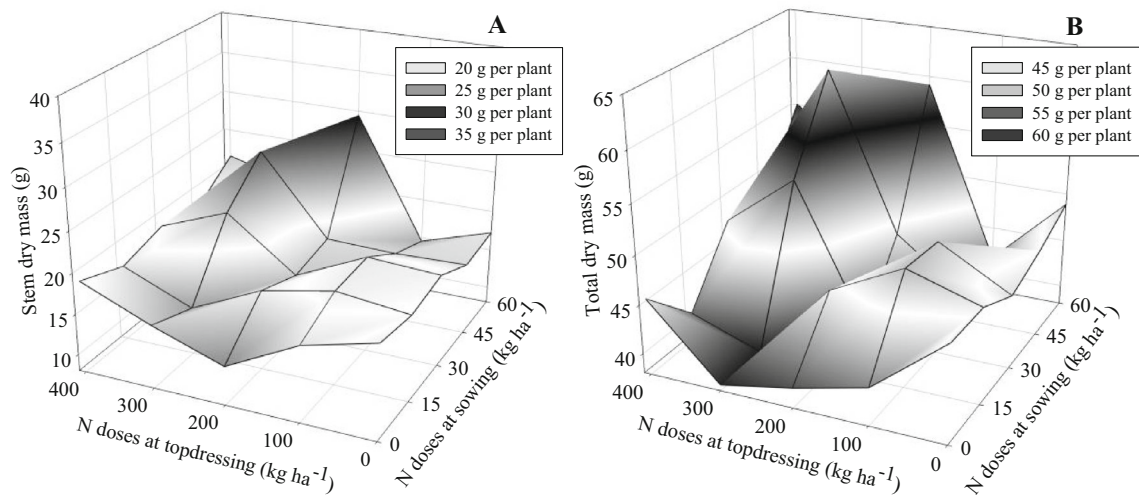


Fig. 5 Stem dry mass and shoot total dry mass of the sweet sorghum at V9 as a function of N doses applied at sowing and topdressing

application of approximately 200 kg N ha^{-1} as topdressing were favored by the initial increments in the development of sorghum (V5) when the highest N dose was applied at sowing (Fig. 5a). Corroborating results obtained for stem dry mass, the increase in N doses at sowing resulted in plants with the greatest increase in total dry mass, 59.2 g per plant , observed in total fertilization with approximately 200 kg N ha^{-1} (Fig. 5b).

The lower dose (100 kg N ha^{-1}) application as topdressing provided a reduction (49%) in stem dry mass, evidencing the importance of an adequate N fertilization at sowing and topdressing for sweet sorghum. Donato et al. (2004) evaluated the chlorophyll content in sugarcane varieties after application of increasing N doses, and observed that the variety with the highest accumulation of biomass also showed the highest content of chlorophyll *a* and *b*. In the present study, the dose of 200 kg N ha^{-1} applied as topdressing promoted the highest chlorophyll *b* (ChlorofiLog[®]) content, total chlorophyll (destructive method), stem dry mass and total dry mass. This shows that the readings taken with the ChlorofiLog[®] properly estimate the extracting chlorophyll content and crop yield at V9.

Biometric and Productivity Analyzes (Harvest)

At harvesting, the interaction between N doses at sowing and at topdressing was not significant for any of the evaluated parameters (plant height, stem diameter, fresh mass production, dry mass production, N accumulation in the plant, stem moisture and broth °Brix). Moreover, stem diameter was not affected by fertilization at sowing or coverage, with average of 21.68 mm (Table 5).

Although plant height showed differences between plots and subplots by the F-test, it did not presented significance for the regression models tested. The average of plant height was 3.16 m . Giacomini et al. (2013), in an experiment carried out in the Central Region of Tocantins, under soils of high fertility, obtained similar results for sweet sorghum cultivars BR 500, BR 504 and BR 505.

The moisture content of the stems at harvesting also did not differ between treatments, and its overall average was 76.8%. Likewise, there was no effect of plots or subplots on dry mass accumulation, with average yield of 20 Mg ha^{-1} . The absence of differences in dry matter productivity, even when the N fertilization is absent, reinforces the hypothesis that the amount of N available in the soil was enough to supply the N demand for sorghum without causing losses of productivity. Obviously, these assumptions need to be tested to draw any conclusions.

As verified in dry mass yield, the total N accumulation was not affected by the evaluated factors, with exported average value of $141.56 \text{ kg N ha}^{-1}$. In a study conducted in Florida (USA), Singh et al. (2012) found similar results to those observed in this study. These authors mentioned that, for each ton of dry matter produced, 6.97 kg of N were extracted. The hybrid Malibu 5010, used in the present study, took 7.07 kg of N per ton of dry matter produced.

The lack of response to N fertilization at the end of the crop cycle can be justified in part by the high organic matter content in the soil, with an estimated supply of 120 kg N ha^{-1} (not measured data). According to Sousa and Lobato (2004), every 10 g dm^{-3} of organic matter can provide 30 kg N ha^{-1} .

Table 5 Plant height (PH), stem diameter (SD), fresh mass production (FMP), dry mass production (DMP), N accumulation in the plant (NAP), ethanol production (EP), stem moisture (SM) and broth °Brix (°Brix) in sweet sorghum at harvest

Total N doses kg ha ⁻¹	PH m	SD mm	FMP mg ha ⁻¹	DMP	NAP kg ha ⁻¹	EP L ha ⁻¹	SM %	°Brix –
0	3.17	21.75	40.4	19.49	138.7	4.0	77.1	13.0
100	3.22	21.74	42.1	19.28	140.2	3.9	77.0	13.0
200	3.15	21.62	45.6	20.28	144.3	4.1	76.4	13.2
300	3.12	21.68	43.7	20.26	142.8	4.1	76.9	13.0
400	3.13	21.61	43.6	20.75	142.1	4.0	76.4	12.9
Means	3.16 ^{ns}	21.68 ^{ns}	43.1 ^{ns}	20.0 ^{ns}	141.6 ^{ns}	4.0 ^{ns}	76.8 ^{ns}	13.0 ^{ns}

^{ns}Not significant at 0.05 probability

Additionally, sweet sorghum is recognized by the high efficiency in the use of N. Barbanti et al. (2006) report that sorghum can produce about 20 Mg ha⁻¹ of dry matter only with the N reserve of the soil. From these values and not considering N losses due to adverse area and climatic conditions, the N release of organic matter from the soil would be able to provide the amount of N exported by the sorghum stems in this study, which represents 70% of the total produced dry biomass (Singh et al. 2012).

The °Brix of broth extracted from the stems was also not altered by the treatments, presenting an average value of 13°. Ethanol production becomes economically feasible when sweet sorghum presents a minimum ART of 12.5%, corresponding to a value of 14.25–14.50 °Brix, being this characteristic directly influenced by the photosynthetic activity (Embrapa 2013).

The °Brix characteristic observed in this study presents a general average below that recommended for sweet sorghum cultivation, which may be associated with the influence of climatic conditions during the growing cycle, i.e., higher rainfall volume, lower physiological demand for water by the plant and consequent lower concentration of soluble solids in the broth. Thus, these data indicate that there is a need for further in-depth investigations under management through planting times within the chosen hybrid, providing information on more stable behavior between growing seasons.

It is also worth mentioning that oversized N doses may prolong the vegetative phase of sorghum, reducing the sugar concentrations in its stems. This is because the accumulation of glucose, fructose and sucrose has been related to the reduction in the photosynthetic activity, especially when the phase of physiological maturation of the grains begins (Jang and Shenn 1994).

As a basis for the production of 43.1 Mg ha⁻¹ of fresh mass, humidity of 76.8% and 13 °Brix, the ethanol production was estimated, which was also not altered by the different N management and presented a mean value of

4000 L ha⁻¹. Emygdio et al. (2011), in an trial with a 40–50 Mg ha⁻¹ shoot yield, obtained ethanol production ranging from 2640 to 3850 L ha⁻¹ for the BRS 506 sorghum cultivar. Even the °Brix having a lower mean than the one considered as adequate, the total fresh mass production of sorghum presented satisfactory yield, and may, in numerical terms, have partly supplied the reduction of the observed sugar contents, showing potential crop to produce biofuel.

These results certainly contributed to minimize the N losses due to the oversized fertilization, guaranteeing greater sustainability of the cultivation system. In addition, the application of high N doses may extend the vegetative growth phase, reducing the sugar concentration in the stems of sorghum (Sawargaonkar et al. 2013).

Conclusions

ClorofiLog[®] can be an useful and reliable tool for the indirect determination of N contents in sorghum leaves, since the results of total chlorophyll *a* and *b* presented very strong correlation ($R = > 0.80$) with the photosynthetic pigments obtained by the destructive method at V9 stage. However, this device cannot be used to N determination in earlier stages of sorghum development. At stage V9, top-dressing N promoted greater increases on the production of dry biomass, the chlorophyll *b* (obtained via ClorofiLog[®]) and total chlorophyll (obtained by the destructive method). However, the gains were not kept until the harvest phase. In soils with high organic matter, the high N availability in the soil tends to level the final production results as in the study conditions that no differences were observed with increasing N doses application up to 400 kg ha⁻¹.

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