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Relative chlorophyll contents in the evaluation of the nutritional status of nitrogen from xaraes palisade grass and determination of critical nitrogen sufficiency index

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ABSTRACT. The nutritional status of xaraes palisade grass with SPAD chlorophyll meter and the critical nitrogen sufficiency index (cNSI) in the dry and rainy seasons were assessed. The experiment was conducted in a completely randomized design with four treatments (50, 100, 200 and 400 kg N ha^{-1}), three replicates and two seasons: dry and rainy. All variables had a high nonlinear relationship with their predictors ($p < 0.001$). Total nitrogen (TN) reached a plateau at doses 262.0 and 514.8 kg of N ha^{-1} and the concentration of TN in the plant was 1.88 and 1.93% respectively during the rainy and dry season. Relative chlorophyll content (RCC) reached a plateau at 46.05 and 53.65 SPAD units in the rainy and dry seasons, respectively. The production of dry matter (DM) showed maximum response to nitrogen fertilization at 209.5 and 229.1 kg N ha^{-1} during the rainy and dry season respectively. The nitrogen sufficiency index (NSI) reached the plateau at 0.85 and 0.99 in the rainy and dry season respectively. All variables showed high linear correlation ($r = 0.71$ to 0.99). The xaraés palisade grass's cNSI is 0.85 and the chlorophyll meter may be used as a nutritional N management tool for the grass.

Keywords: fertilization, *Urochloa brizantha*, chlorophyll meter, nutritional status, SPAD.

Teor relativo de clorofila na avaliação do status nutricional de nitrogênio do capim-xaraés e determinação do índice de suficiência do nitrogênio crítico

RESUMO. Objetivou-se avaliar o estado nutricional do capim-xaraés utilizando o clorofilômetro SPAD e estabelecer o índice de suficiência crítico (ISNc) nas épocas das águas e seca. O experimento foi conduzido em um delineamento inteiramente casualizado com quatro tratamentos (50, 100, 200 e 400 kg N ha^{-1}), três repetições, duas épocas: águas e seca. Todas as variáveis apresentaram alta relação não linear com seus preditores ($p < 0,001$). O nitrogênio total (NT) alcançou o platô na dose de 262,0 e 514,8 kg de N ha^{-1} e a concentração de NT na planta foi 1,88 e 1,93% no período das águas e seco, respectivamente. O teor relativo de clorofila (TRC) atingiu o platô a 46,05 e 53,65 unidades SPAD no período das águas e seca, respectivamente. A produção de matéria seca (MS) apresentou máxima resposta a adubação nitrogenada na dose de 209,5 e 229,1 kg N ha^{-1} na época das águas e seca, respectivamente. O índice de suficiência de nitrogênio (ISN) atingiu o platô em 0,85 e 0,99 na época das águas e secas respectivamente. Todas as variáveis apresentaram alta correlação linear (r= 0,71 a 0,99). O ISNc do capim-xaraés é 0,85 e o clorofilômetro pode ser utilizado como ferramenta de manejo nutricional de N para essa gramínea.

Palavras-chave: adubação, *Urochloa brizantha*, clorofilômetro, estado nutricional, SPAD.

Introduction

Nitrogen (N) is the most required mineral for the growth and biomass production of grasses. Moreover, it is highly important for dry matter increase of pastures in the production system of ruminants. However, the excessive use of the mineral may increase its disposal by leaching, with the subsequent pollution of surface and underground water resources and the emission of nitrous oxide (Ladha et al., 2005).

Chlorophyll meters have been developed to decrease financial and environmental liabilities through the excessive use of nitrogen. The device measures the relative quantity of chlorophyll in leaves. Since the intensity of the color green is greatly related to the plant's nitrogen rates, its nutritional state may be predicted (Piekkielek & Fox, 1992). When fertilized (control) and non-fertilized sectors are measured, the nitrogen sufficiency index (NSI), or rather, the relation between the chlorophyll rates in the control and in the non-fertilized segment, may be assessed. When NSI reaches the plateau, denominated critical nitrogen sufficiency index (cNSI), no additional response to production is expected (Samborski et al., 2009).

According to Samborski et al. (2009), cNSI ranges between 0.71 and 0.99 and has to be established for different crops (Esfahani et al., 2008; Hawkins et al., 2007; Maia et al., 2012), requiring its establishment for each crop and even for its varieties (Hussain et al., 2000). However, such information is not available for tropical grasses and the calculation of cNSI for forage grasses will be performed by chlorophyll meter so that nitrogen use in the fertilization of pastures could become more efficient.

Xaraes palisade grass has high forage production and has been employed as a forage alternative. Studies with Soil Plant Analysis Development (SPAD) chlorophyll meter are not extant for the xaraes palisade grass and to assess its nutritional status. The relationship between the chlorophyll's relative rate (CRR), measured by the chlorophyll meter, and the variables involved in the nitrogen's nutritional status should be evaluated so that SPAD could be employed as a tool for the grass's nutritional management.

Current assay establishes the cNSI for the xaraes palisade grass for nitrogen doses during the dry and rainy seasons and also the co-relationship of variables involved in the assessment of the nutritional status of nitrogen with those obtained by the chlorophyll meter.

Material and methods

The experiment was performed at the Faculty of Agrarian and Veterinary Sciences of the Universidade Estadual Paulista "Júlio de Mesquita Filho" in Jaboticabal, São Paulo State, Brazil, in the Forage Culture Section of the Animal Science Department, at 21º15'22" S and 48º18'58"W, altitude 595 m. Assay started in September (dry period) 2011 and lasted till January (rainy period) 2012. Figure 1, shows mean temperatures and rainfall during the period. A 24 m² enclosure with pasture grass *Urochloa brizantha* cv. Xaraés was used, divided into 12 plots of 2 m² each. At the start of the experiment, the area's red dystrophic latosol (0 - 20 cm layer) comprised the following chemical characteristics: pH ($CaCl_2$): 5.3; organic matter (g dm^{-3}): 20; P resin (mg dm^{-3}): 22; K (mmol$_c$ dm^{-3}): 1.6; Ca (mmol$_c$ dm^{-3}): 48; Mg (mmol$_c$ dm^{-3}): 13; H+Al (mmol$_c$ dm^{-3}): 34; SB(mmol$_c$ dm^{-3}): 62.6; T (mmol$_c$ dm^{-3}): 96.6; V (%): 65.

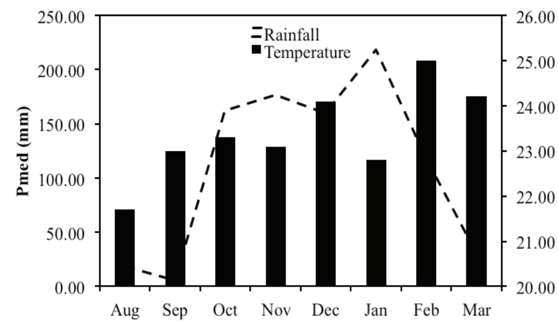

Figure 1. Mean rainfall and mean temperature during the experimental period, from August 2011 to March 2012.

The experiment was evaluated in a totally randomized design, with four doses of Nitrogen (50, 100, 200 and 400 kg ha^{-1}), three replications and two evaluation periods, the rainy and the dry seasons. Prior to the start of the assay, the area was mowed up to 15 cm by a mechanical mower and the area was fertilized by a single dose of urea, as nitrogen source, after three days of grass recovery. Amounts of potassium (K_2O) and phosphorus (P_2O_5) were maintained respectively at 75 and 80 kg ha^{-1}. The area was irrigated during the dry season the day before and after fertilization to lessen loss by volatilization.

A 95% light interception, obtained by dossel analyzer device (AccuPAR Model LP – 80 PAR/LAI, Decagon devices®) was employed as a criterion for the interruption of forage growth and collection of produced mass samples. Two 0.25 m² samples from each plot were collected, dried in a forced air circulation at 55ºC for 72 hours and weighed to obtain the forage dry matter per hectare (DM, kg ha^{-1}). Samples of dry plants were ground and total nitrogen rates (TN%) were measured by macro Kjedahl method (AOAC, 2005).

CRR was measured by chlorophyll meter Minolta SPAD-502 Plus (Konica Minolta Sensing Americas Inc., Ramsey, MI, USA) with 15 measurements of CR Roer plot on harvest in different plants at the third mid-section of the last leaf totally expanded between the edge and the main nerve of the leaf. Measurement was taken at 10 am to avoid reading error due to water droplets on the leaves. The CRR of each treatment provided NSI, the quotient between CRR rates in the leaf for each treatment and leaf's CRR rates obtained in the plot for the highest N dose (400 kg N ha^{-1}) (Blackmer &

Schepers, 1994). The NSI was adjusted according to the nitrogen rate difference from the economic optimum nitrogen rate (dose of each treatment – the nitrogen rate difference from the economic optimum nitrogen rate obtained by maximum DM production= NDEONR, kg of nitrogen ha^{-1}) to find the cNSI (Peterson, 1993).

All variables were adjusted to the quadratic plateau (QP) model according to Equation 1:

$$Y = L + U (R-X)^2 \quad \text{if } X < R; \quad (1)$$

$$Y = L, \quad \text{if } X \geq R;$$

where:

Y = dependent variable; L = asymptotic response of the model; U = inclination up to break point; R = break point at axis X; X = independent variable (N doses or NDEONR).

The QP model was adjusted to the variables by NLIN procedure of SAS (2004). The model's parameters were estimated by Gauss-Newton's minimum square method. Linear co-relation test between variables was also performed with CORR procedure.

Results and discussion

All the adjusted variables displayed high non-linear relationship with their predictors ($p < 0.001$), with R^2 between 0.79 and 0.94. TN had maximum concentration in the plant at dose 262.0 (kg N ha^{-1}) during the rainy season, whereas dose 514.8 (kg N ha^{-1}) was reached during the dry one. TN concentrations in the plateau were 1.889 and 1.939% respectively for the rainy and dry seasons (Table 1 and Figure 2). In fact, a rise in N dose increased TN accumulation in the plant. Accumulation was stabilized as from N doses 262.0 and 514.8. CRR had a quadratic increase up to doses 287.2 and 350.0 kg of N ha^{-1} and reached the plateau at 46.05 and 53.65 SPAD units respectively for the rainy and dry seasons (Table 1; Figure 3).

Similar to TN, the CRR's plateau was lower for the rainy period. However, the plant had the lowest TN concentration during the dry period at a dose below 400 kg N ha^{-1}. TN rate became higher only from this dose till it reached the plateau. The above behavior suggested that N absorption and accumulation was affected by climate conditions since it was the only apparent change in the proposed treatments. Water deficit occurred since the rainfall rate was low during the dry period (Figure 1). Consequently, N conducted by the soil's reduced solution (mass flow) was consequently low and thus less absorbed by the roots. Loss by volatilization of applied N was another factor that may have contributed towards a lower N absorption and accumulation in the plant. In fact, during this period, rainfall rates were low, nitrogen was not conducted to the roots and volatilization occurred. The above is corroborated by the fact that, in spite of the plant's TN during the dry period showed a quadratic behavior, the concavity was visually mild, seemingly close to a linear behavior. This evokes the volatilization behavior reported by Martha Jr. et al. (2004), proportional to the quantity applied, or rather, each applied dose had a lost section, unavailable to the plant.

Since CRR was higher during the dry period at all fertilization levels, a great TN use in the production of chlorophyll during the period is suggested since solar irradiation during this time of the year is normally less. Such behavior may be explained by the fact that plants growing under low irradiation increase their chlorophyll amounts (Martuscello et al., 2009). They increase the efficiency of light absorption so that production could be maintained. Since RCC reached the plateau in the two periods, it may be registered that even if N is added by fertilization, the plant will not produce more chlorophyll.

Table 1. Parameters of the quadratic plateau that describe response standards of xaraes palisade grass to nitroginated fertilization during the rainy and dry seasons.

Y[a]	X	L		U		R		P-value	R^2
		Estimated	SE	Estimated	SE	Estimated	SE		
TN	N (rainy)	1.889	0.043	-0.00001	0.000005	262.0	48.08	<0.0001	0.90
TN	N (dry)	1.939	0.041	-0.0000038	0.000001	514.8	56.05	<0.0001	0.94
CRR	N (rainy)	46.05	1.336	-0.00019	0.000191	287.2	77.76	0.0006	0.81
CRR	N (dry)	53.65	1.080	-0.00017	0.000172	350.0	52.10	<0.0001	0.93
DM	N (rainy)	7042.8	172.6	-0.0660	0.0512	209.5	60.3	0.0006	0.81
DM	N (dry)	6229.3	253.3	-0.0649	0.0510	229.1	71.1	0.0007	0.79
NSI	NDEONR (rainy)	0.85	0.025	-0.0000042	0.000001	55.99	1.12	0.0008	0.80
NSI	NDEONR (dry)	0.99	0.013	-0.0000012	0.000001	283.9	83.72	<0.0001	0.94

[a]Y: dependent variable; X: independent variable; L: asymptotic response of the model (rupture point of axis Y); U: inclination up to rupture point; R: rupture point at axis X; TN (%) total nitrogen; N (kg ha^{-1}), applied nitrogen; CRR (SPAD units): chlorophyll relative rate; DM (kg ha^{-1}): dry matter; NSI: nitrogen sufficiency index; NDEONR (kg ha^{-1}): nitrogen rate difference of the economic optimum nitrogen rate; SE: standard error.

The above behavior is known as photosynthetic maturity and was previously reported for forage grasses (Batista & Monteiro, 2007; Lavres Junior et al., 2010; Lavres Junior & Monteiro, 2006). According to Lavres Junior et al. (2010), the behavior suggests the accuracy of CRR and TN in the vegetal tissue as a nutritional assessment tool of nitrogen in tropical grasses. Hussain et al. (2000) report the importance of the chlorophyll meter for an adequate cNSI. If the plateau is not reached, it will not be possible to know the maximum rate that RCC may reach.

DM has the maximum response to nitroginated fertilization at doses 209.5 and 229.1 kg N ha^{-1} respectively during the rainy and dry seasons (Table 1; Figure 4). Maximum production in the above doses reached 7042.8 and 6229.3 kg of DM per ha^{-1} respectively during the rainy and dry seasons. N is a mineral highly required by the grass for its growth and thus grasses respond to high doses of the mineral. However, current assay reveals that xaraes palisade grass responded up to 209.5 kg N ha^{-1} during the rainy season and 229.1 kg N ha^{-1} during the dry one. Therefore, the doses above those established in current assay may not increase the xaraes grass's response to productivity. According to Crowder and Chheda (1982), maximum response is linked to several factors, especially genetic and climatic ones that benefit N absorption.

NSI responded to fertilization till it reached the plateau in 0.85 and 0.99 respectively during the rainy and dry seasons, with cNSI of xaraes grass at 0.85 (Table 1; Figure 5). Consequently, xaraes palisade grass fails to respond to additional fertilization above the NSI rate. During the dry period the cNSI reached 0.99 and was not detected within the dose intervals used in current assay. Therefore, nitrogen was not the limiting factor during this period but rather growth and production restricted by low temperatures and frequent rainfall indexes during the period.

In the several crops in which cNSI was obtained, all occurred within the 0.99 and 0.89 interval (Samborski et al., 2009). However, the crops where such rates were detected are high N-requiring crops, such as corn (Hawkins et al., 2007) and rice (Hussain et al., 2000). These rates are the first to be reported in the literature for tropical grasses. Due to the lack of such information to use SPAD-502 Plus as management tool of nitrogenized fertilization of xaraes grass, the estimates of maximum response to fertilization have been probably overestimated. When 0.95 were used as cNSI for the crop, the required N levels would be much higher than that established in current assay. Therefore, cNSI should be established for each species and even for different cultivars, as suggested by Hussain et al. (2000) who used 0.90 as the cNSI for rice when the number indicated would be 0.95 (established for corn) and had a higher efficiency in the use of N through fertilization. During the dry season, cNSI was 0.99, higher than that detected in the rainy period. The above increase may be linked to a higher RCC concentration during the period, as Figure 2 shows. Therefore, cNSI varies according to the period of the year. During the dry season, the variable had the lowest co-relationship with DM production when compared with that for the rainy season. The use of chlorophyll meter as a nutritional management tool of xaraes palisade grass is not indicated for the dry season.

Table 2 demonstrates results of the analysis of linear co-relationship between the variables related to the nutritional status of xaraes grass. As a rule, all variables provided a high positive co-relationship since *r* ranged between 0.71 and 0.99, especially during the rainy period in most variables. However, NSI and RCC are the variables with the highest coefficient of co-relationship, perhaps due to the fact that NSI was obtained from RCC. In its turn, RCC displayed a high co-relationship with TN. Certain assays have dealt with this relationship (Costa et al., 2008; Lavres Junior & Monteiro, 2006) and, according to Costa et al. (2008), the high relationship between N contents and chlorophyll rates measured by the chlorophyll meter, called RCC, may be a promising manner of evaluating the nutritional status of N in xaraes grass. The lowest coefficients of co-relation were detected when the test was performed with DM production. Nevertheless, coefficients were higher than 0.71, which is a satisfactory co-relationship. According to Lavres Junior et al. (2010), high RCC relationship with DM production is an alternative for the monitoring of the nutritional status since N is the main component of the chlorophyll molecule (Mengel et al., 2001). As a rule, the variables analyzed in current assay are reliable for the monitoring of the nutritional status of N of xaraes grass since they all displayed high co-relationship.

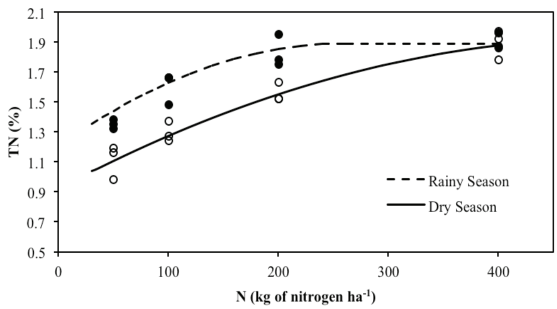

Figure 2. Response of xaraes palisade grass at total nitrogen (TN) concentration with regard to N dose during the rainy (●) and dry (○) seasons.

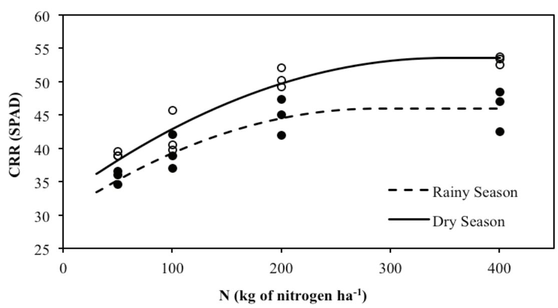

Figure 3. Response of xaraes palisade grass to chlorophyll relative rate (CRR) with regard to nitrogen dose during the rainy (●) and dry (○) seasons.

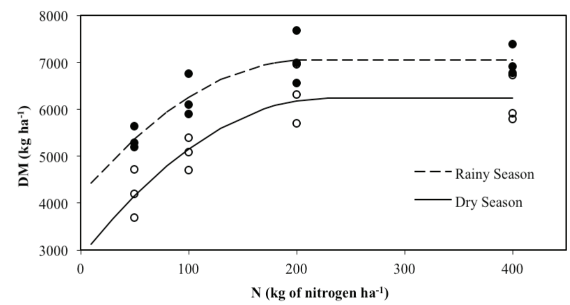

Figure 4. Response of xaraes palisade grass to the production of dry matter (DM) with regard to nitrogen dose during the rainy (●) and dry (○) seasons.

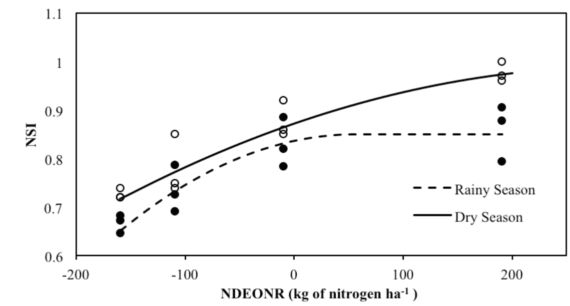

Figure 5. Response of xaraes grass to nitrogen sufficiency index (NSI) with regard to the nitrogen rate difference of the economic optimum nitrogen rate (NDEONR) in the rainy (●) and dry (○) seasons.

Table 2. Coefficient of simple linear co-relationship and significance of the test between the variables related to the nutritional status of N of xaraes grass and the employment of chlorophyll meter.

Item	TN		CRR		NSI		DM	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
TN	1.000	1.000	0.929 < 0.001	0.937 < 0.001	0.964 < 0.001	0.941 <0.001	0.715 0.008	0.799 0.002
CRR			1.000	1.000	0.945 < 0.001	0.998 < 0.001	0.839 < 0.001	0.842 < 0.001
NSI					1.000	1.000	0.788 0.002	0.827 < 0.001
DM							1.000	1.000

TN: total nitrogen; CRR: chlorophyll relative rate; DM: dry matter; NSI: nitrogen sufficiency index.

Conclusion

The variables demonstrate a high non-linear relationship and co-relationship with the nutritional status of nitrogen in xaraes grass. Therefore, the chlorophyll meter may be employed as a tool to evaluate the nutritional status of N which should be administered by using cNSI 0.85 for the rainy period. The chlorophyll meter must not be employed during the dry season.

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