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Indicators of the nitrogen nutritional status of sugarcane submitted to nitrogen rates

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Abstract: Diagnosis of the nutritional status of sugarcane is an important complementary tool for management of fertilization. The objective of this study was to evaluate nitrogen nutritional status of sugarcane submitted to nitrogen rates by conventional method and alternatives indicators. The experiment was conducted in a greenhouse with the IACSP95 5000 cultivar, grown in 20 L pots containing sand and vermiculite. The treatments consisted of six nitrogen rates (0, 5, 10, 20, 40, 80 mg dm^{-3} of N) using urea as a source. From ten to fourteen days after fertilization, nine productive, nutritional and physiological parameters were evaluated and correlated (shoot dry weight, root dry weight, N content in leaves, total shoot N content, total shoot N accumulation, nitrate content in leaves, NR activity, chlorophyll index in type 0 leaf and chlorophyll index in type +1 leaf). All nutritional, physiological and productive indicators, with the exception of the root biomass, were affected by the variation in the nitrogen supply. There was a high correlation between nitrate reductase enzyme activity and chlorophyll content with leaf N content, suggesting a high potential to indicate the nutritional status of sugarcane.
Keywords: chlorophyll, enzyme activity, nitrogen fertilization, plant nutrition.

Introduction

Recommendation of nitrogen (N) based on the amount that sugarcane plants really need is fundamental to avoid productivity decrease due to lack of the nutrient or loss of technological quality caused by excess of the N (Madhuri et al., 2011). Diagnosis of the nutritional status is an important tool for the correct management of nitrogen fertilization (Muñoz-Huerta et al., 2013). This can be achieved based on leaf concentrations, or alternatively by chlorophyll meters or measurement of enzyme activity (Römheld, 2012).

The main form of N available to plants is nitrate (NO_3^-). Once absorbed, the ions are reduced to nitrite (NO_2^-) by the plant, starting the process of assimilating N (Masclaux-Daubresse et al., 2010). The reduction of NO_3^-, performed by the enzyme NR, is the step that limits the incorporation of N in plants (Lillo, 2008), and consequently their productive potential. In general, the production of biomass is influence by the level of N acquired, which in turn can be influence by the N rate applied (Ishikawa et al., 2009; Lofton et al., 2012).

It is known that nitrogen fertilization affects NR activity in sugarcane. Both Abayomi et al. (1988) as Silveira e Crocomo (1990) observed directly proportional increases, reflected in greater production of stalks, suggesting the possible existence of a positive relation between NR activity and N rate. Therefore, methods that measure nitrate reductase (NR) activity (Römheld, 2012), for example, have potential for purpose of nutritional evaluation. However, further research is necessary to support this idea.

Diagnosis based on total N levels in the leaves (Raij e Cantarella, 1997) is still the most common technique for sugarcane. However, studies have shown that alternative diagnostic methods that quantify ionic fractions of N, such as nitrate, or specific nitrogen compounds, in particular chlorophyll, that are active in plants' metabolism also can reflect the nutritional status (Amaral e Molin, 2011; Robinson et al., 2011; Bassi et al. 2018), with the advantage of being faster than the conventional method (leaf N content). In this context, the objective of this study was to evaluate nitrogen nutritional

status of sugarcane submitted to nitrogen rates by conventional method and alternatives indicators.

Methods

The experiment was conducted in a greenhouse at the Faculty of Agricultural and Veterinary Sciences (FCAV), UNESP in Jaboticabal, Sao Paulo state, Brazil, between January and April 2012. The treatments consisted of six N rates (equivalent to 0, 5, 10, 20, 40 and 80 mg dm^{-3} of N), applied on the substrate surface in the form of a urea solution, diluted in 200 mL of water, when the plants started to show visual symptoms of N deficiency (starting 86 days after transplantation). A blocks randomized design with four replicates was used. At the time of sampling, it was impossible to analyze all at once, so each block was collected in one day, for 4 consecutive days (from 10 to 14 days after N application).

Each pot (20 dm^3) contained two sugarcane plants (IACSP95-5000 cultivar). The seedlings were produced from December 2011 to January 2012 from mini-ratoons (5 cm in length) obtained from the Sao Paulo State Agribusiness Technology Agency (APTA), which were germinated in cups with volume of 0.5 dm^3 filled with sand having medium granulometry (0.5 mm). Thirty-five days after germination, when the plants were about 6 cm tall (from the base to the insertion of leaf +1, which is the first leaf from top to bottom that presents a visible dewlap), the seedlings were transplanted to pots, filled with a mixture of sand with medium granulometry and commercial vermiculite in a ratio of 2:1 (v/v), respectively.

The substrate in the pots had the following chemical properties: pH in $CaCl_2$ = 6.5; OM = 3 g dm^{-3}; P (resin) = 5 mg dm^{-3}; K = 0.5 $mmol_c.dm^{-3}$; Ca = 5.0 $mmol_c.dm^{-3}$; Mg = 64 $mmol_c.dm^{-3}$; H+Al = 7.0 $mmol_c.dm^{-3}$; SB= 69.5 $mmol_c.dm^{-3}$; CEC = 76.5 $mmol_c.dm^{-3}$; and V = 91%. The plants were grown in sand and vermiculite to subject them to nitrogen deficiency.

The extrapolations for correction of fertility were based on the indications of Raij e Cantarella (1997) for field conditions, with incorporation of 90 and 75 mg dm^{-3} of P_2O_5 and K_2O, supplied in the form of single superphosphate and potassium chloride, respectively. The N was applied afterward, and the K_2O was applied in the proportion of 2/3 in the pot substrate at time of transplanting (50 mg dm^{-3}).

From the transplantation, a Hoagland solution was applied weekly to the substrate to supply the other macro and micronutrients, containing S, Mg, Ca, Fe, Zn, B, Mn, Cu and Mo. The remaining K rate (1/3) was applied in the substrate 30 days after transplanting, in the form of a solution of KCl (25 mg dm^{-3} of K_2O).

Ninety-six days after transplanting, nine variables were evaluated: plant height; Falker chlorophyll index (FCI) in 0 and +1 type leaves; nitrate reductase activity (type 0 leaf); shoot dry weight; leaf N content (type +1 leaf); total shoot N content; shoot N accumulation; and leaf nitrate content (type 0 leaf), according to the sequence detailed below.

The chlorophyll index was measured on each evaluation day in type 0 and +1 leaves. The readings were obtained starting at 10:00 a.m., and 12 readings were taken for each pot, using a ClorofiLOG® model CFL 1030 electronic meter.

The leaves immediately above type +1 leaves, i.e., type 0, were collected to determine the nitrate reductase activity (NRa). The NRa was measured in type 0 leaves, cut into square sections with sides of 0.5 cm, without ribs. Each sample consisted of 500 mg of leaf tissue. The *in vivo* method used was according to the standard described by Santos et al. (2014), and all the measurements were conducted in triplicate. The NRa was expressed in μmol g^{-1} h^{-1} of NO_2^-.

After the basal cut, the plant material was washed with a detergent solution and then rinsed with deionized water. The roots were separated from the substrate by they same washing technique. All the samples were placed in an oven with closed circulation at 65 °C until reaching constant weight.

The leaf concentration of N was determined by the Kjeldahl method described in Embrapa (2009) and $N\text{-}NO_3^-$ by Salicylic Acid Method for nitrate determination in extracts from dried plant tissue (Cataldo et al., 1975).

The data were submitted to analysis of variance by the F-test. When significant, regression analysis was performed, using the Sisvar statistical program (Ferreira, 2011). The correlation between the variables was performed with the PAleontological Statistics – PAST software (Hammer et al., 2001).

Results and discussion

The nitrate reductase activity (NRa) increased as a function of N rate according to a quadratic model (Figure 1A), similar to the observation of Abayomi et al. (1988) after supplying 100 kg ha^{-1} of N to two sugarcane genotypes. In the present study, the N rate that caused the highest NRa was 48.5 mg dm^{-3}, which is equivalent to 97 kg ha^{-1} of N. Thus it can be inferred that this activity in sugarcane plants is affected by variations in the supply of nitrogen.

The enzyme uses nitrate (NO_3^-) as its substrate, nitrate which in turn also regulates its synthesis and controls post-synthesis (Lillo, 2008; Konishi e Yanagisawa, 2011). Thus, the low activity in the treatment without N (Figure 1A) might have been due to the low availability of $N\text{-}NO_3^-$ in the medium, which triggered a low quantity of the enzyme in the plant.

In contrast, adding urea in the culture medium caused an increase in the enzyme activity (Figure 1A). It is known that the process of nitrification from ammonia produced by the hydrolysis of urea is relatively rapid (Soares et al.,

2012). Therefore, the NO_3^- ions must have been available in the medium until 12 days (±2) after application of urea (moment of analysis), and their absorption triggered the enzyme transcription, increasing its concentration in the cells, reflected in the increased activity.

There was a decrease in enzyme activity at the higher N rates (Figure 1A), indicating that the uptake of N in quantities greater than necessary for the plants can impair enzyme activity. In a study of fennel plants, Rad et al. (2013) observed that nitrogen supplementation beyond the necessary level suppressed the biosynthesis pathway of NR, consequently impairing uptake of N. Our results corroborate this finding.

Although the leaf nitrate level presented a very low coefficient of determination (R^2 = 0.35), we observed a tendency for a quadratic fit, according to which the nitrate level declined in function of rising N rates, behavior inverse to that observed for NRa (Figure 1A). The lowest value observed was 0.35 mg g^{-1} with the application of 47 mg dm^{-3} of N (Figure 1B), when the NR activity was the highest (Figure 1A). Therefore, it can be stated that this rate of 47 mg dm^{-3} provided the best induction of the enzyme by the substrate (Masclaux-Daubresse et al., 2010), below which the incorporation of nitrate in the plant is limited by the availability of substrate and above which it is limited by the reduction of enzyme activity.

The opposite was observed without application of N, where the nitrate content of the leaves was greatest (0.58 mg g^{-1}), as can be seen in Figure 1B, indicating that the absence of the enzyme to reduce nitrate induced greater leaf concentration. These observations suggest that nitrate absorbed at the first moment acted to induce synthesis of NR (Konishi e Yanagisawa, 2011), and as it was absorbed in greater quantity, it also induced greater enzyme synthesis. Also in studies of sugarcane, both Silveira e Crocomo (1990) and Ishikawa et al. (2009) observed that even application of high N rates did not cause significant increases in the content or accumulation of nitrate in the leaves, suggesting that in conditions adequate for maximum synthesis of the enzyme, the ions are reduced immediately on being absorbed.

The Falker chlorophyll index (FCI) of leaves 0 and +1 increased with a quadratic fit in function of N rate. The lowest chlorophyll content was observed in the absence of N application (Figures 1C and 1D). The readings reached 50.74 and 51.73 respectively, with application of 51 and 47 mg dm^{-3} of N.

The chlorophyll concentration in sugarcane is generally associated with nitrogen nutrition of plants (Bassi, Menossi e Mattiello, 2018), so it can be used indirectly to estimate the concentration of N (Muñoz-Huerta et al., 2013). Here we evaluated leaves 0 and +1 and noted very similar results (Figures 1C and 1D). We also observed that the behavior of the chlorophyll index was very similar to the leaf N content (Figure 2A). Amaral e Molin (2011) stated that more studies are necessary about leaf sampling for correct assessment, but the authors confirmed the possibility of applying chlorophyll measurement as a tool to manage fertilization of sugarcane, as also indicated by our results (Table 1).

The low chlorophyll levels observed can be related to the low NR activity also observed at low N rates or absence of N application (Figure 1A). According to Garkar et al. (2011), NR activity is the main controller of the assimilation of NO_3^- taken up from the soil, and if the quantity of N assimilated is low, the synthesis of chlorophyll is limited, causing lower readings to be observed.

The leaf N content increased quadratically in function of rising N rates, reaching a peak of 18.66 g kg^{-1} with use of 53 mg dm^{-3} of N (Figure 2A).

Despite the amplitude of the concentration range, the figures fell short of the range considered adequate by Raij e Cantarella (1997). Due to questions related to the sample, we noted in general that when no N was applied, the levels were very low. On the other hand, with application of N, the levels increased, but did not reach the sufficiency range presented by Raij e Cantarella (1997), which is from 18 to 25 g kg^{-1}. Nevertheless, this does not necessarily mean the plants were malnourished, because the experimental setup did not allow sampling the plants with ideal age or at the age indicated by those authors.

The behavior of the leaf N concentration was similar to that of the NR activity (Figure 1A) and identical to the chlorophyll reading (Figures 1C e 1D) and accumulation of N (Figure 2C). This indicates that under the conditions studied, these parameters are good indicators of the N nutritional status of sugarcane plants. According to Muñoz-Huerta et al. (2013), the use of more than one diagnostic tool is important, since a determined parameter can present advantages over others, such as greater sensitivity.

We observed a linear relation between the shoot N content and rate applied. Without nitrogen application, the shoot content was 3.45 g kg^{-1} and as the N rate increased, the shoot concentration also did, until peaking at 5.28 g kg^{-1} with the N rate of 80 mg dm^{-3} (Figure 2B). This linear behavior, in contrast to the quadratic relation for leaf N content (Figure 2A), indicates that the N absorbed was incorporated in greater proportions in the plant tissue other than in the diagnostic leaves.

The data on accumulation of N in the aerial part in function of N rates were best fit by a quadratic curve. The highest accumulation occurred at the N rate of 56 mg dm^{-3} and was 48.5 mg $plant^{-1}$ (Figure 2C). An increase of N accumulation after application of N to sugarcane was also observed by Ishikawa et al. (2009), Madhuri et al. (2011) and Robinson et al. (2011).

The shoot dry weight (SDW) increased quadratically in function of N rate applied, but with a small coefficient of determination (R^2 = 0.47) (Figure

2D), reaching 10.24 g plant^{-1} with application of 43 mg dm^{-3} of N. The increase of SDW after application of N on sugarcane was also observed by Robinson et al. (2011) in an experiment with a nutrient solution.

With application of the highest rate, accumulation of SDW was impaired (Figure 2D). Lofton et al. (2012) reported the same observation in assessing the production of sugarcane stalks. The occurrence of losses with the use of high N rates can be associated with the nitrate absorption and/or the toxic effects of ammonium. In the first case, a possible excessive absorption of nitrate could explain, at least in part, the reduction in the SDW in the greater rate because the process of reduction of the nitrate consumes a lot of energy of the plant (Silveira e Crocomo, 1981). In the second case, since ammonium is the form preferred by sugarcane plants (Robinson et al., 2011), the plants might have absorbed a large part of the N in this form, and might not have had the capacity to assimilate all the ammonium absorbed, probably inducing toxicity.

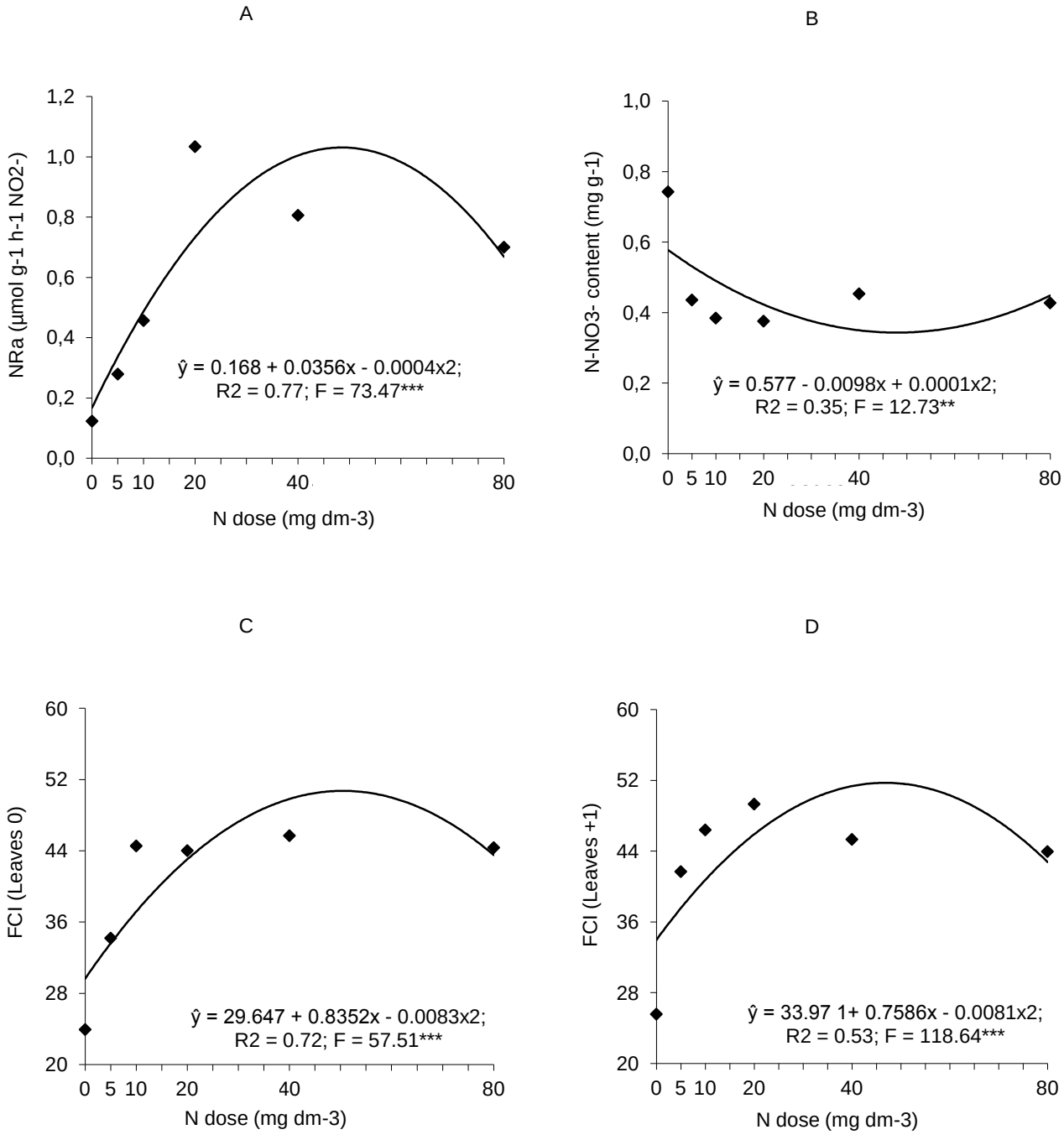

Figure 1. Physiological parameters assessed in sugarcane leaves (IACSP95-5000 cultivar), in function of N rate; (A) NR activity in type 0 leaves; (B) nitrate content in type 0 leaves; (C) and (D) Falker chlorophyll indices in type 0 and +1 leaves, respectively. *** and ** = significant at 0.1% and 1% probability.

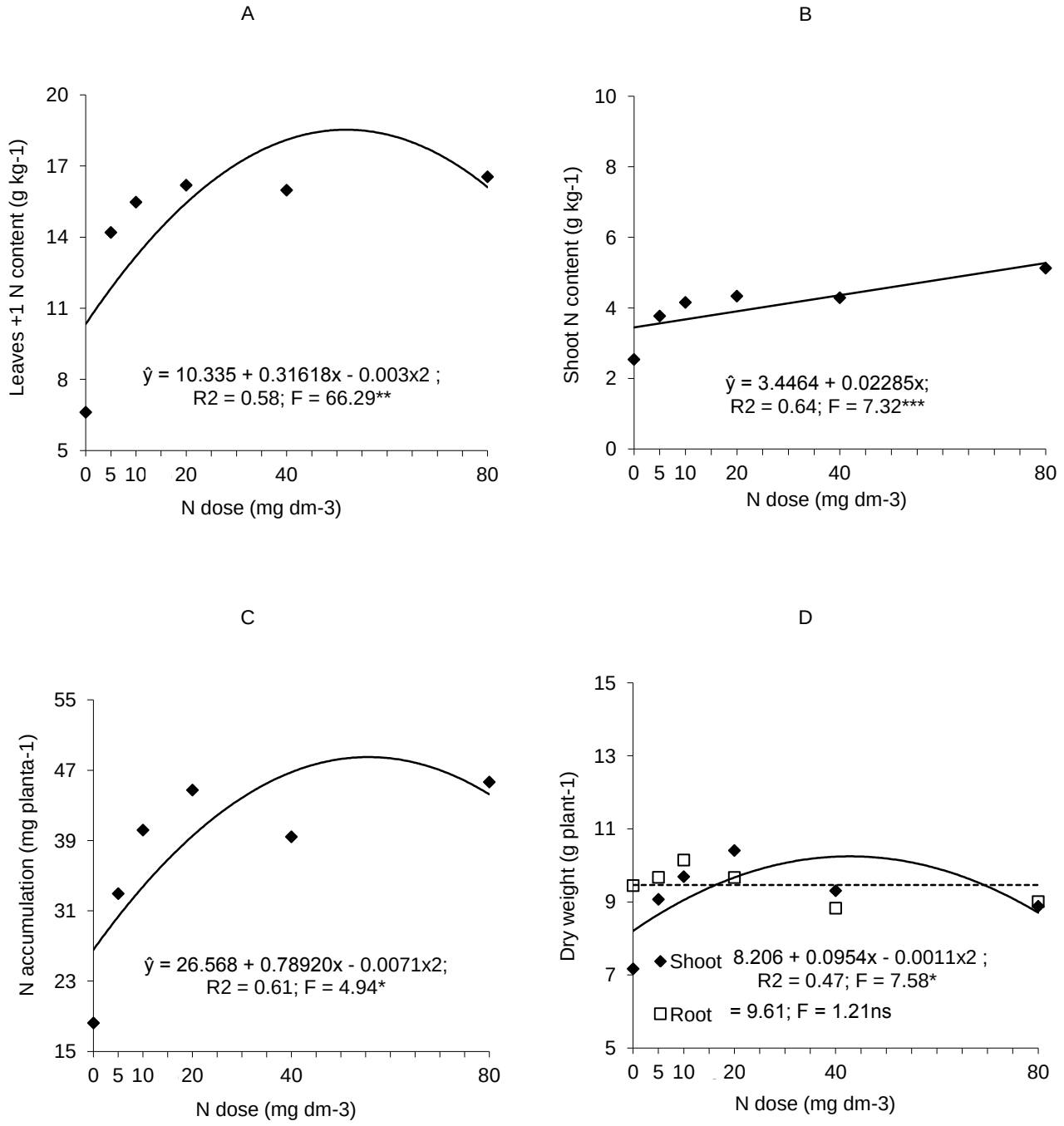

Figure 2. Nutritional and productive parameters assessed in sugarcane leaves (IACSP95-5000 cultivar) in function of N rate; (A) total N content in +1 leaves (B) total shoot N content; (C) total shoot N accumulation; (D) shoot and root dry weight *** ** and * = significant at 0.1%, 1% and 5% probability, respectively; ^{ns} – not significant.

The root dry weight was not affected by the N rate, presenting an average value of 9.46 g plant^{-1} (Figure 2D). Probably the presence of N induced the plants to stop producing roots. Besides this, the short interval between application of N and harvesting of the plants (12 days ±2) might not have been sufficient to induce a significant increase in root growth.

High negative correlation coefficients were observed between Nitrate content and Falker

chlorophyll indices in type +1, total N content in +1 leaves and total shoot N accumulation (Table 1). Nitrate content was also associated negatively, but with moderate degree, to the NRa, FCI-0, SDW and TSNC. High positive coefficients were observed when were associated NR activity with total N content in +1 leaves and with Falker chlorophyll indices in the two types of leaves evaluated. Other strong correlations were also observed (FCI-0 with FCI-1, FCI-0 with TNCL, FCI-1 with TNCL, FCI-1 with TSNA, TNCL with TSNA and TSNC with TSNA). Root dry weight did not correlate with any of the variables and TSNC also did not correlate with SDW. The associations between the remaining variables were moderate and positive.

Due to the high positive correlations between the indirect measurement of chlorophyll and nitrate reductase activity (alternative indicators) with leaf N content (standard method), it can be inferred that there is potential for the use of these indicators as a tool to evaluation of the Sugarcane N nutritional status. Thus, the results suggest positive perspectives for the progress of research aimed at the development of alternative methods for nutritional diagnosis.

Table 1. Simple linear correlation coefficients (R) and the significances between productive, nutritional and physiological parameters evaluated in sugarcane fertilized with nitrogen rates.

	NRa	FCI-0	FCI-1	SDW	SDR	TNCL	TSNC	TSNA
NRa								
FCI-0	0,732**							
FCI-1	0,727**	0,871**						
SDW	0,489*	0,503*	0,534**					
SDR	-0,130^{ns}	0,022^{ns}	-0,089^{ns}	0,335^{ns}				
TNCL	0,700**	0,870**	0,905**	0,592**	-0,024^{ns}			
TSNC	0,426*	0,444*	0,566**	0,108^{ns}	-0,129^{ns}	0,602**		
TSNA	0,599**	0,588**	0,706**	0,514*	0,009^{ns}	0,756**	0,900**	
NO_3^-	-0,553**	-0,677**	-0,813^{ns}	-0,551**	-0,003^{ns}	-0,824**	-0,556**	-0,697**

NR activity in type 0 leaves (RNa); Falker chlorophyll indices in type 0 (FCI-0) and +1 (FCI-1) leaves; shoot dry weight (SDW) and root dry weight (RDW); total N content in +1 leaves (TNCL); total shoot N content (TSNC); total shoot N accumulation (TSNA); Nitrate content in type 0 leaves (NO_3^-); **, * and ^{ns} – significantly at 1%, 5% and non-significantly by the t-test, respectively.

Conclusions

All nutritional, physiological and productive indicators, with the exception of the root biomass, were affected by the variation in the nitrogen supply. There was a high correlation between nitrate reductase enzyme activity and chlorophyll content with leaf N content, suggesting a high potential to indicate the nutritional status of sugarcane.

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