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RESEARCH ARTICLE

Nutritional and Physiological Response of Sugarcane Varieties to Nitrogen Fertilization in a Haplic Cambisol

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Abstract In order to assess the nutritional and physiological response of sugarcane varieties COLPOSCTMEX 05-223, COLPOSCTMEX 06-039, RD 75-11, COLPOSCTMEX 06-271, MEX 69-290, COLPOSCTMEX 06-2362, MEX 68-P-23 COLPOSCTMEX 472, ATEMEX 96-40 and CP 72-1210 to nitrogen doses of 0, 120 and 180 kg ha^{-1}, foliar N, P and K, chlorophyll content, photosynthesis rate (A), transpiration rate (E) and stomatal conductance (gs) were recorded for 150 days. The study was conducted in fields of sugar factory Azsuremex (IA), Tabasco, Mexico, during the growing season of 2014/2015, under rainfed conditions with Haplic (silty–eutric–calcaric) Cambisol. No variation was observed among the sugarcane varieties studied, in terms of the nitrogen doses applied, foliar N and P. Foliar K concentrations were deficient in all varieties (0.55–0.80 %). The dose of nitrogen did not affect chlorophyll concentration or photosynthesis rate in any variety, but it had an effect on gs and E. Under conditions of increasing photosynthetically active radiation, the variety ATEMEX 96-40 was performed better than the varieties COLPOSCTMEX 05-223 and RD75-11B in terms of A, gs, internal CO_2 concentration (Ci) and transpiration rate (E).

Keywords Nutritional diagnosis · Nitrogen fertilizer dose · Photosynthesis · Stomatal conductance · Sugarcane varieties

Introduction

In Mexico, sugarcane is grown in 15 out of 31 states, with an average yield of 74 t ha^{-1}. Tabasco, the highest sugar production region in Mexico, is located in the Gulf of Mexico where the average sugarcane yield is 60 t ha^{-1}. The alternating flood and drought periods during the crop growing stage are main factors that affects sugarcane yield in Tabasco which consequently lead to a delay in fertilizer application and, results in delayed crop maturity (Salgado-García et al. 2008).

Given the hike in the price of fertilizers and a growing need to preserve the environment, the use of nitrogen fertilizers in sugarcane through proper guidance needs to be rationalized (Salgado et al. 2003; Franco et al. 2011).

For sugarcane fields that provide mill able canes to sugar factory Azsuremex in Tabasco, Mexico, fertilizer doses have been prescribed for N, P and K without considering the sugarcane variety grown (Salgado-García et al. 2010), which may potentially affect the fertilizer requirement in the region. Several studies have shown that the sugarcane varieties differ as to their ability to absorb and use the nutrients naturally available from soil as well as those available through application of fertilizers (Thangavelu and Rao 2014; Madhuri et al. 2011). The ability of sugarcane varieties to grow and produce high yields with low nutrient

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levels depends on their nutrient-use efficiency. Efficient varieties may have either lower nutrient needs or a greater capacity to uptake and utilize the nutrients from soil (Srinivas et al. 2003). The nutrient-use efficient sugarcane varieties can be identified using yield, N and P indices (Kumar et al. 2005).

Nutritional deficiencies may affect the synthesis of proteins, enzymes and photosynthetic pigments; the deficiency in potassium, a nutrient that acts directly on stomatal adjustment, which may affect the rate of photosynthesis (Galon et al. 2013). Laboratory and field data have shown a decrease of about 25 % in gross canopy photosynthesis and nearly 30 % in net photosynthesis, even under conditions of standard crop fertilization. This decrease is reduced when supplementary fertilization is applied (Allison et al. 1997). Nitrogen is essential for the synthesis of amino acids, chloroplasts and chlorophyll. Its application to soil increases the number and size of leaves, which results into an increase in sugarcane photosynthetic activity and yield (Cha-Um and Kirdmanee 2008). In sugarcane, the demand for nitrogen and the response of the crop vary according to the physiological development stages, which in turn are affected by genetic or environmental differences (Kumara and Bandara 2001).

In Mexico, most investigations on sugarcane nutrition have focused on its effect on sugarcane yield and sugar production while little attention has been given on the physiological response of sugarcane varieties to different doses of nitrogen fertilizer and its relationship with the plant's nutritional status. This information supplements the agronomic information required for improving the selection of the varieties cultivated in each region of the country, specifically as regards to their response to different fertilizer doses. For the above reasons, the objectives of this work was to evaluate the nutritional and physiological response of sugarcane varieties to the application of different doses of nitrogen fertilizer.

Materials and Methods

The experiment was conducted under rainfed conditions during 2014/2015 cane plant cycle, at Azsuremex (SFA) sugar factory, Tenosique, Tabasco, Mexico, which is located at 17° 25′ N and 91° 24′ W, at an altitude of 60 metres above sea level with mean annual temperature 26 °C, rainfall 1595 mm and relative humidity 83 %. Soil of the field is Haplic (silty–eutric–calcaric) Cambisol with pH 7.2, and nutrient availability of N, P_2O_5 and K_2O is 38, 83 and 185 kg ha^{-1}, respectively (Salgado-García et al. 2010). IA is famous for the manufacturing of brown sugar, as well as molasses and filter cake as by-products of cane.

The experimental design comprises of split plots, where the main plot is divided into three N levels, namely 0, 120 and 180 kg ha^{-1}, with urea as N source. The dose of nitrogen fertilizer is applied in a single application supplemented with 60 kg P_2O_5 and 100 kg K_2O per hectare, with SPT and KCL as sources when the crop was 2 months old (Salgado-García et al. 2010). After the plantation of 10 months, the subplots corresponded to the 10 varieties of sugarcane, namely COLPOSCTMEX 05-223, COLPOSCTMEX 06-039, RD 75-11, COLPOSCTMEX 06-271, MEX 69-290, COLPOSCTMEX 06-2362, MEX 68-P-23 COLPOSCTMEX 472, ATEMEX 96-40 and CP 72-1210. The experimental plot consisted of four rows each measuring 12 linear metres. The experiment included four replicates.

The experimental field was prepared, and furrows were opened at a spacing of 1.3 m. Planting was performed manually on 12 January 2014.

After 2 and 5 months of plantation, weeds were controlled by applying herbicide picloram + 2.4 amine and atrazine as pre-emergence and also as post-emergence. In addition, a crop pass was conducted with hooks, along with hilling and manual weeding.

When the crop was four months old, the fourth leaf (leaf 4) was collected from each treatment. The leaf blade was dried at 60 °C for 24 h and milled in a 2-mm mesh size Wiley mill for N, P and K analysis (Jones et al. 1991). On the same date, four readings per treatment were taken from leaf 4 with a SPAD-502 handheld chlorophyll meter, and the chlorophyll content was determined according to Moran (1982). For chlorophyll content, six discs of leaf 4 were obtained per treatment using a 1-cm^2 circular drill. Three discs were placed in a 10-mL test tube containing 5 mL of *N*, *N*-dimethylformamide, protected from light, and were transported to the laboratory at 4 °C. In the laboratory, the incubation was continued at room temperature under dark to complete 24 h. Absorbance was read at 647 and 664 nm on a Thermo Scientific model Multiskan go UV–visible spectrophotometer. The three remaining discs were used for recording dry weight after drying at 60 °C. A correlation was performed between SPAD-502 readings and chlorophyll content.

After 150 days of planting, photosynthesis rate (µmol CO_2 m^{-2} s^{-1})(A), stomatal conductance [gs (mol m^{-2} s^{-1})], internal CO_2 concentration [Ci (µmol mol^{-1})] and transpiration rate [E (mmol H_2O m^2 s^{-1})] were measured in leaf 4 from ten plants in the middle row for each treatment. Five varieties were chosen in which two varieties (ATEMEX 96-40, RD 75-1) cultivated in the area due to their good agronomic performance, a new variety (COLPOSCTMEX OR5-223) that is under testing in the area and two varieties (MEX 68-P23, MEX 69-290) extensively grown in the southeast of Mexico. All measurements were made in triplicate and recorded using a handheld photosynthesis system (LI-COR model LI6400) fitted with a blue/red light source (LI6400-02B) at a photosynthetically active radiation of 1500 µmol m^{-2} s^{-1}. Furthermore, in the three varieties that showed better agronomic performance without N restrictions, the response to photosynthetically active radiation (PAR) was determined. Response curves were constructed where PAR was reduced gradually from 2000 to 0 µmol m^{-2} s^{-1} (2000, 1500, 1000, 500, 200, 100, 50, 20 and 0 µmol m^{-2} s^{-1}) in varieties: ATEMEX 96-40, RD 75-11 and COLPOSCTMEX 05-223, with a fertilizer dose of 180 kg N ha^{-1}.

Statistical Analysis

For the nutritional analysis, SPAD units, chlorophyll and physiological parameters, analysis of variance was conducted for split-plot design. The Tukey's multiple comparison test was conducted using the statistical package SAS version 9.0.

Results and Discussion

Nutritional Diagnosis

No significant differences in foliar N concentration were observed with respect to nitrogen dose, but the differences among sugarcane varieties were found statistically significant (Table 1). Similar results were reported by Robinson et al. (2008), who stated that the application of 140 kg N ha^{-1} (high dose) in sugarcane crop resulted in a significant increase in N in stalk tissues—but not in leaves, suggesting that N was not transported to leaves but stored in the stalk, which might affect the quality of sugar if N levels are excessive (Yang et al. 2013). These findings, however, differ from the results reported by Kumara and Bandara (2001), where the application of nitrogen fertilizer significantly increased the concentration of foliar nitrogen in sugarcane varieties grown in Sri Lanka, although with a differential varietal response. The treatment with no N application led to a 28 % reduction in this variable than to the treatment with application of 200 kg N ha^{-1}, as recorded in the sampling performed 150 days after planting.

Foliar N concentrations showed no significant differences among sugarcane varieties, with an average N concentration of 1.79 %. This indicates that the requirement for N has been fulfilled by application of fertilizers. Different standards for interpreting foliar N concentrations are available in the literature. According to Jones et al. (1991), the average N content of the varieties under study has been considered low if it is in the range of 1.6–1.9 % N, while for Halliday and Trenkell (1992) the N concentration is deemed adequate if it is in the range of 1.5–1.7 % N. In the present case, the ploughing up of the previous crop and the incorporation of its wastes might have facilitated the mineralization of organic matter in soil, with the consequent release of N in a sufficient quantity to meet the crop's demand, since foliar N concentration at the three N doses applied was similar; hence, the effect of nitrogen fertilization has been minimized.

Table 1 Foliar content of nitrogen, phosphorus and potassium in ten sugarcane varieties with different nitrogen rates of nitrogen application

Varieties	Means variety (%)		
	N	P	K
COLPOSCTMEX 06-039	1.89a	0.22a	0.63ab
COLPOSCTMEX 06-271	1.74a	0.21a	0.79a
COLPOSCTMEX 06-474	1.86a	0.21a	0.62ab
COLPOSCTMEX 06-2362	1.70a	0.21a	0.51b
COLPOSCTMEX 05-223	1.85a	0.20a	0.80a
ATEMEX 96-40	1.84a	0.23a	0.64ab
RD 75-11	1.73a	0.19a	0.64ab
MEX 69-290	1.78a	0.19a	0.58b
MEX 68-P23	1.80a	0.20a	0.64ab
CP 72-1210	1.72a	0.20a	0.55b
Means rate (N)	1.79NS	0.20NS	0.64NS
C.V (%)	7.8	14.3	19.3
Rate N (N)	0.71NS	0.18NS	0.17NS
Variety (V)	0.05*	0.1NS	0.01**
Interaction (N × V)	0.97NS	0.32NS	0.95NS
SMD (N)	0.08	0.01	0.07
SMD (V)	0.21	0.04	0.19

† Means with the same letter in the row or column are statistically equal Tukey ($P \leq 0.05$). *NS* not significant, * significant, ** highly significant

Similar to foliar N, no significant differences were observed for foliar P with respect to the nitrogen dose and sugarcane variety, nor for the N × V interaction; this can be expected, since the dose of P_2O_5 was identical for all treatments (Table 1). The average foliar P was 0.20 % which, according to Jones et al. (1991), is deemed moderate (0.18–0.30 %), whereas Halliday and Trenkell (1992) considered it adequate (0.18–0.22 %). This result indicates that the dose of phosphorus fertilizer recommended for the study area (60 kg P_2O_5) and applied in the present investigation is adequate (Salgado-García et al. 2010). In the case of K, a highly significant difference is observed between varieties, and no significant differences were found related to the nitrogen dose and the N × V interaction (Table 1).

Based on foliar K content, the 10 varieties that were considered for this study can be classified into two groups with respect to their ability to assimilate soil's K. The average leaf K concentration in the 10 varieties was 0.64 %, which is found low as compared to the optimal foliar K values (1.0–1.8 %), required for optimal crop development (Jones et al. 1991). K deficiency can interfere with the opening and closing of stomata causing increased water consumption and less efficient assimilation of carbon necessary for the formation of sugars in the leaves; therefore, crop growth is reduced. This suggests that the dose of 100 kg ha^{-1} of K_2O applied was not sufficient to meet the demand for this nutrient among the sugarcane varieties studied and needs to review the dose of K applied given the content of this element in the Cambisol soil. Thus, soil type showed a low K content, 0.12 cmolc kg^{-1} and high Ca and Mg concentrations, which could have competed with K for absorption by roots (Salgado-García et al. 2010).

Physiological Parameters

There were highly significant differences in SPAD units and total chlorophyll content between sugarcane varieties, but non-significant differences were observed regarding N doses and the N × V interaction. The coefficient of variation of 25.6 indicates a high variability for this parameter by SPAD units; on the other hand, a coefficient of 13.76 % was obtained for chlorophyll concentration (Table 2). The variety COLPOSCTMEX 472 shows the highest content of SPAD with 31.27 units while the variety MEX 69-290 displayed the lowest content with 18.4 units; the remaining varieties showed intermediate values between 22.21 and 27.95 SPAD units. These results are consistent with levels observed in the Coimbatore varieties, where SPAD readings ranged from 14 to 33 units for varieties Co 86032 and Co 87025, respectively (Radhamani et al. 2015).

Córdova-Gamas et al. (2016) reported non-significant differences in chlorophyll activity with different fertilizer treatments for the plant crop of variety MEX 79-431 with an average chlorophyll activity of 27 SPAD units; these authors suggested that the N content was satisfactory. In contrast, Jaroenseng et al. (2010) determined that a reading of 34 SPAD units in sugarcane leaves is regarded as an indication of sufficient N content, whereas readings lower than 34 are considered deficient N concentration for the sugarcane varieties grown in Thailand.

With regard to the chlorophyll content, although variation was observed with respect to varieties, the ranking of varieties did not match the ranking based on SPAD units. The correlation between the two measurements was 0.31 ($P \leq 0.01$). The variety COLPOSCTMEX 06-039 showed the highest total chlorophyll content (1523.5 mg g^{-1} dry weight) while the variety MEX 68 P23 displayed the lowest value. The chlorophyll levels observed exceed the average value of 0.649 mg g^{-1} dry weight reported for the Coimbatore varieties (Radhamani et al. 2015). In contrary Kumara and Bandara (2001) found that the application of nitrogen fertilizer led to a significant increase in leaf chlorophyll content in sugarcane, while the treatment with no N application resulted in a 10 % drop in chlorophyll content compared to the treatment with application of 200 kg N ha^{-1} in the sampling performed 150 days after planting.

Table 2 SPAD units and leaf chlorophyll content of ten sugarcane varieties with different rates of nitrogen application

Varieties	Means variety	
	SPAD units	Total chlorophyll mg g^{-1} dry weight
COLPOSCTMEX 06-039	24.54ab	1523.50a
COLPOSCTMEX 06-271	26.23ab	1159.32bcd
COLPOSCTMEX 06-474	31.27a	1324.77ab
COLPOSCTMEX 06-2362	22.21ab	1014.37 cd
COLPOSCTMEX 05-223	27.95ab	1404.33ab
ATEMEX 96-40	27.73ab	1316.48ab
RD 75-11	27.32ab	1218.46bc
MEX 69-290	18.40b	1026.41 cd
MEX 68-P23	22.78ab	957.24d
CP 72-1210	24.72ab	1156.09bcd
Means rate (N)	25.31NS	1206.57NS
C.V (%)	25.6	13.7
Rate N (N)	0.67NS	0.26NS
Variety (V)	0.01**	0.01**
Interaction (N × V)	0.87NS	0.18NS
SMD (N):	4.03	103.7
SMD (V):	10.07	259.2

† Means with the same letter in the row or column are statistically equal Tukey ($P \leq 0.05$). *NS* not significant, ** highly significant

Prior to the determination of A, gs and E, light-response curves were obtained for varieties ATEMEX 96-40, COLPOSCTMEX 05-223 and RD 75-11 at 180 kg N ha^{-1}, (at no N deficiency). To note, maximum A levels are attained from 1000 µmol m^{-2} s^{-1} in all varieties (Fig. 1).

The varieties ATEMEX 96-40 and COLPOSCTMEX 05-223 showed higher A and E values compared to RD 75-11 (Fig. 1a and d). The intracellular CO_2 (Ci) concentration in the three varieties is found inversely related to photosynthetically active radiation (PAR); that is, higher PAR figures were associated with lower Ci values, and vice versa (Fig. 1c), related to CO_2 uptake for photosynthesis. The differences observed between sugarcane varieties might be due to plant architecture, where plants with a lower A for a high PAR might have had a higher number of

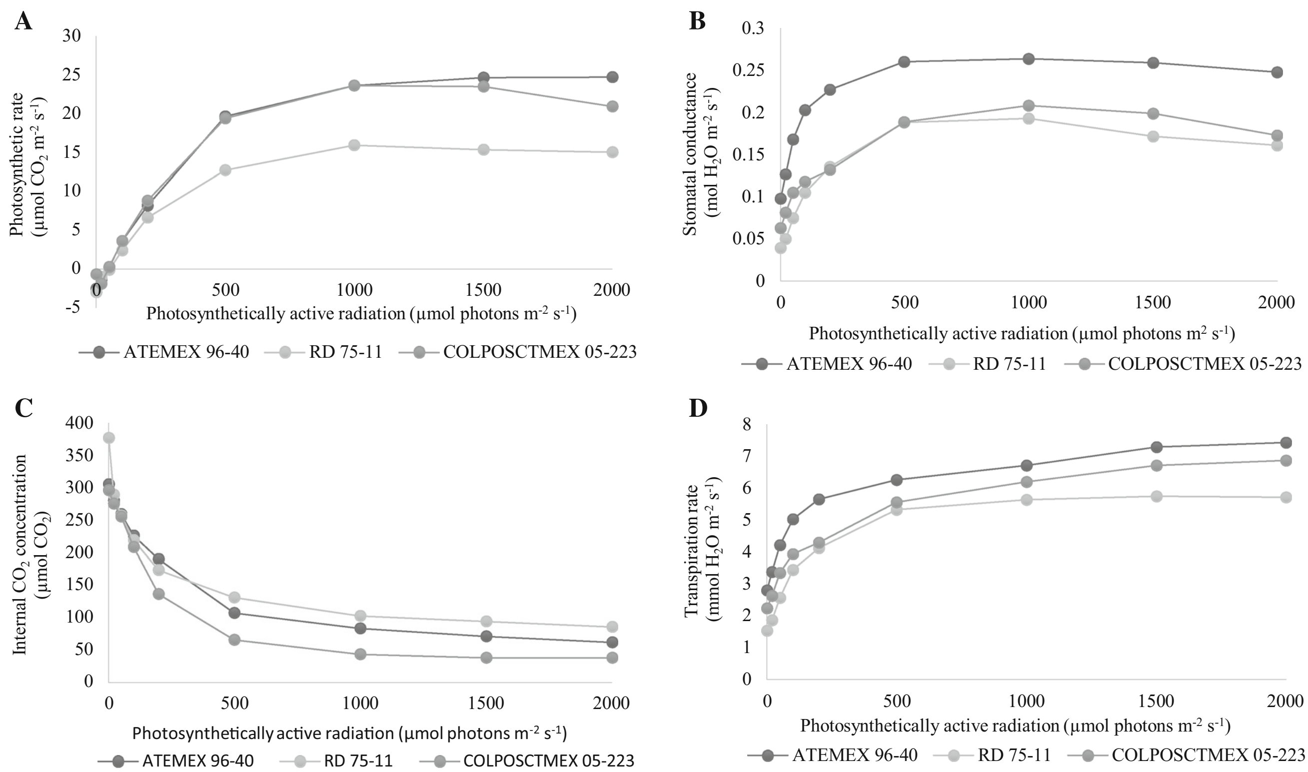

Fig. 1 Light response curves of some physiological parameters in three sugarcane varieties. **a**. Photosynthetic rate, **b** stomatal conductance, **c** internal CO_2 concentration and **d** transpiration rate

leaves in the shade. When assessing the response to light availability of different sugarcane varieties at three canopy levels, Marchiori et al. (2010) noted that the variety IACSP95-3028, whose upper canopy showed a high exposure to light, showed an increase in the rate of photosynthesis and improved vegetative growth by increasing tillering, leaf area and biomass accumulation in leaves and stems.

Similar to the varietal response to N and total chlorophyll content in sugarcane leaves from the different fertilization treatments, no significant differences were observed in photosynthesis rate (A) with respect to the effect of nitrogen dose (N), but the opposite is true for the effect of varieties (V) and the N × V interaction (Table 3). These results contrast with the findings reported by Kumara and Bandara (2001), who pointed out that the application of nitrogen fertilizer significantly increased A in sugarcane varieties irrigated at 10-day intervals up to the 11th month of the experiment. The treatment with no N application led to a 18 % reduction in A compared to the treatment with application of 200 kg N ha^{-1} in the sampling performed 150 days after planting.

The variety ATEMEX 96-40 showed the highest A, with 21.94 µmol CO_2 m^{-2} s^{-1}, followed by varieties COLPOSCTMEX 05-223 (17.21 µmol CO_2 m^{-2} s^{-1}) and RD 75-11 (16.71 µmol CO_2 m^{-2} s^{-1}); the varieties that displayed the lowest A were MEX 68-P23 (14.23 µmol CO_2 m^{-2} s^{-1}) and MEX 69-290 (13.96 µmol CO_2 m^{-2} s^{-1}). These values are similar to those observed by Irvine (1975) in 30 varieties of Australian sugarcane (16–24 µmol CO_2 m^{-2} s^{-1}) and by De Silva and De Costa (2009) in eight sugarcane varieties grown under rainfed conditions in Sri Lanka (10.4 to 27.3 µmol CO_2 m^{-2} s^{-1}). A rise in A has been observed resulting from irrigation that is related to higher gs. The values of A ranged between 23.0 to 28.1 µmol CO_2 m^{-2} s^{-1} in different sugarcane varieties grown under irrigation (De Silva and De Costa 2009). Higher A values have been reported for the variety RB835486 (44 µmol CO_2 m^{-2} s^{-1}) (Sage et al. 2014) and for 10 Brazilian varieties (Ferreira et al. 2011). In the present study, the variety COLPOSCTMEX 5-223 showed the highest A level at 180 kg N ha^{-1}. Moreover, varieties RD 75-11 and MEX 68-P23 showed peak A levels at 120 kg N ha^{-1} (Table 3), which is the recommended nitrogen dose in the study area (Salgado-García et al. 2010).

Unlike A, gs was more sensitive to the N dose applied (Table 3). The 120 and 180 kg N ha^{-1} doses were associated with the highest gs (0.22 mol m^{-2} s^{-1}). The lack of application of nitrogen reduced gs up to 18 % in 180 kg N ha^{-1} as compared with 120 kg N ha^{-1}. Varieties were grouped as follows: ATEMEX 96-40 was the variety with

Table 3 Photosynthetic rate (A, µmol Co_2 m^{-2} s^{-1}), stomatal conductance (g_S, mol m^{-2} s^{-1}), internal Co_2 concentration (Ci, µmol Co_2) and transpiration rate (E, mmol m^{-2} s^{-1}) to 150 days after sowing in sugarcane varieties with different nitrogen rates

Varieties	Means variety			
	A	gs	Ci	E
ATEMEX 96-40	21.43a	0.26a	87.47b	7.32a
COLPOSCTMEX 05-223	17.06b	0.21b	90.86ab	6.30b
RD 75-11	16.61b	0.20b	90.64ab	5.93b
MEX 68-P23	14.21c	0.17c	92.71ab	5.36c
MEX 69-290	13.87c	0.16c	93.64a	5.04c
Means rate N (kg ha^{-1})				
0	15.88b	0.18b	84.76b	6.21a
120	16.78ab	0.21a	93.70a	6.34a
160	17.11a	0.21a	95.31a	5.34b
C.V (%)	25.2	28.5	16.6	18.5
Rate N (N)	0.07NS	0.01**	0.06NS	0.04*
Variety (V)	0.01**	0.01**	0.01**	0.01**
Interaction (N × V)	0.01**	0.01**	0.01**	0.01**
SMD (N):	1.1	0.01	4.0	0.2
SMD (V):	1.6	0.02	6.09	0.4

† Means with the same letter in the row or column are statistically equal Tukey ($P \leq 0.05$). *NS* not significant, * significant, ** highly significant

the highest gs (0.27 mol m^{-2} s^{-1}); varieties COLPOSCTMEX 05-223 and RD 75-11 showed intermediate gs levels; and varieties MEX 68-P23 and MEX 69-290 showed the lowest gs (0.17 mol H_2O m^{-2} s^{-1}), showing a high correlation with A. In the five varieties studied, gs ranged from 0.17 to 0.27 mol m^{-2} s^{-1}, similar to the range observed under rainfed conditions in eight sugarcane varieties (0.18–0.29 mol m^{-2} s^{-1}) 159 days after planting (De Silva and De Costa 2009). These are lower than gs values reported by Ferreira et al. (2011) in 10 varieties of Brazilian sugarcane (0.19–0.50 mol m^{-2} s^{-1}). In contrast, Marchiori et al. (2010) found that the photosynthesis rate (A) and stomatal conductance (gs) in leaves over daylight hours were similar in varieties IACSP93-2060, IACSP95-3028 and IACSP95-5000, with A ranging from 13 to 15.5 µmol CO_2 m^{-2} s^{-1} and gs from 0.09 to 0.11 mol m^{-2} s^{-1}. The N × V interaction indicates that an interaction exists between gs in a given variety and the dose of N applied (Table 3).

Similar to gs, the transpiration rate (E) of the sugarcane varieties studied was affected by the nitrogen dose applied, sugarcane variety and N × V interaction (Table 3). No significant differences were observed between the doses of 0 and 120 kg N ha^{-1}, but the dose of 180 kg N ha^{-1} led to a 15 % drop in E relative to the low nitrogen dose. The E variation among the varieties studied was found between 5.05 and 7.37 mmol H_2O m^{-2} s^{-1}, which was higher than the range reported by Ferreira et al. (2011) for 10 varieties of Brazilian sugarcane (2.8–5.8 mmol H_2O m^{-2} s^{-1}) and by De Silva and De Costa (2009) for eight sugarcane varieties grown under rainfed conditions (1.4–2.1 mmol H_2O m^{-2} s^{-1}), and similar to the values E obtained for these same varieties under irrigation (3.7–6.0 mmol H_2O m^{-2} s^{-1}).

Conclusions

The sugarcane varieties evaluated in the study showed no variation with respect to the dose of nitrogen applied in terms of N, P, K, chlorophyll content and photosynthesis rates at 150 days, whereas gs and E varied between sugarcane varieties and N doses applied. The foliar concentration of N and P was adequate, contrary to K, which was deficient. This suggests that the dose of K would be recommended for the Haplic (silty–eutric–calcaric) Cambisol at SFA needs to be reviewed to avoid the competition of K with Ca and Mg for radical absorption. After 150 days, the variety ATEMEX 96-40 displayed high A levels in response to the increase in PAR and also higher total chlorophyll, A, gs and E values in response to nitrogen fertilization.

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