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Physiology and Productivity of Sugarcane as Affected by Nitrogen Applied Via Subsurface Drip Irrigation

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Abstract: Most of sugarcane cultivation in Brazil is carried out under non-irrigated conditions, restricting crop yield due to lack of water. Nitrogen (N) fertilization via subsurface drip irrigation can promote the physiological processes of plants and increase the stalk and sugar yield of sugarcane. This present study aimed to evaluate irrigation and N use in sugarcane ratoon, and assess how this might affect physiological parameters and crop yield. We used cultivar SP80-3280 under subsurface drip irrigation and non-irrigated systems and two N levels (0 and 150 kg N ha^{-1}). Irrigation and N fertilization rendered positive effects for stalk and sugar yields, as well as for plant physiological parameters. Our results showed that irrigation had positive effects over physiological parameters of sugarcane plants, generating great increase in water use efficiency for stalk and sugar production. It was also observed that the supplementary application of 25% water at the highest water deficit period for the crop has increased stalk yield in 84-94% and sugar yield in 86-100%. Nitrogen fertilizer showed increased efficiency in irrigated crop as well, promoting 84% gain in stalk yield. Therefore, subsurface drip irrigation is a great technique to increase crop yield and/or reduce the effects caused by drought.

Keywords: *Saccharum* spp., water deficit, nutrient, physiological traits, water use efficiency, yield.

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1 Introduction

Globally Brazil is leading sugarcane (*Saccharum* spp.) producer and during 2015-16 it was cultivated on 9.13 m ha. South-Central region of the country occupied 88.5% of total area under sugarcane cultivation specifically states of São Paulo (51.4%), Goiás (9.6%) and Minas Gerais (8.6%) (CONAB, 2016). Sugarcane cultivation is concentrated in the South-Central region of Brazil mainly due to climate conditions, more specifically, because these regions have a rainy and warm summer, promoting vegetative growth, and cold and dry winters, promoting sugarcane maturation (Carvalho et al., 2015).

The rainy and hot season (November to March) is very important for plant development, for it is during this period that high vegetative growth occurs (Jadoski et al., 2010). During this period, high temperature of air, greater light and water availability favor sprouting, tillering and shoot growth, and, above all, stalk development and elongation, which is the industry's primary interest product. From an industrial perspective, the cool and dry season (June to September) is very important for plant aging, since reduced air temperature and water availability in the soil induce plant accumulation of sucrose in stalks (Toppa et al., 2010; Bonnett, 2013).

Therefore, the greater the plant growth in warm and rainy season, the larger stalk production per area and consequently the greater the sucrose production, for the greater stalk and internode number per area, the higher plant reserve volume to accumulate sucrose in stalks (van Heerden et al., 2010; Jones et al., 2015). In the central region of São Paulo state, annual precipitation is close to 1.400 mm, with 75% of this precipitation occurring between October and March. Therefore, any factor reducing plant growth (specifically in the hot and rainy season) will promote stalk yield reduction, as well as the sucrose production reduction. In São Paulo state, the average yield in the 2016/17 of sugarcane cultivated only under rainfall conditions was 75.5 t ha^{-1}, while under irrigated conditions the yield of stalks may exceed 130 t ha^{-1} (Silva et al., 2014; CONAB, 2016).

Among several environmental factors, lack of water is the main factor that limits agricultural crop yield. Agriculture sector is leading water consumer in Brazil. In Parana River basin average water withdrawal increased by more than 300%, from 100 m^3 s^{-1} 2006 to 311.4 m^3 s^{-1} in 2010. However, only 25% increase was recorded for industrial water withdrawals. Many regions of Brazil are experiencing chronic water shortage, mainly due to climate change induced reduction in precipitation (Scarpare et al., 2016).

Practically all sugarcane produced in the state of São Paulo is grown under non-irrigated conditions, which tradition is rooted in the paradigm that sugarcane irrigation is not economically viable in the soil and climatic conditions of the state (Silva et al., 2014). However, in recent years some works with irrigated sugarcane crop have shown sugarcane yield increase between 31.5 to 145 % (Gava et al., 2010; Oliveira et al., 2011; Rhein and Silva, 2017).

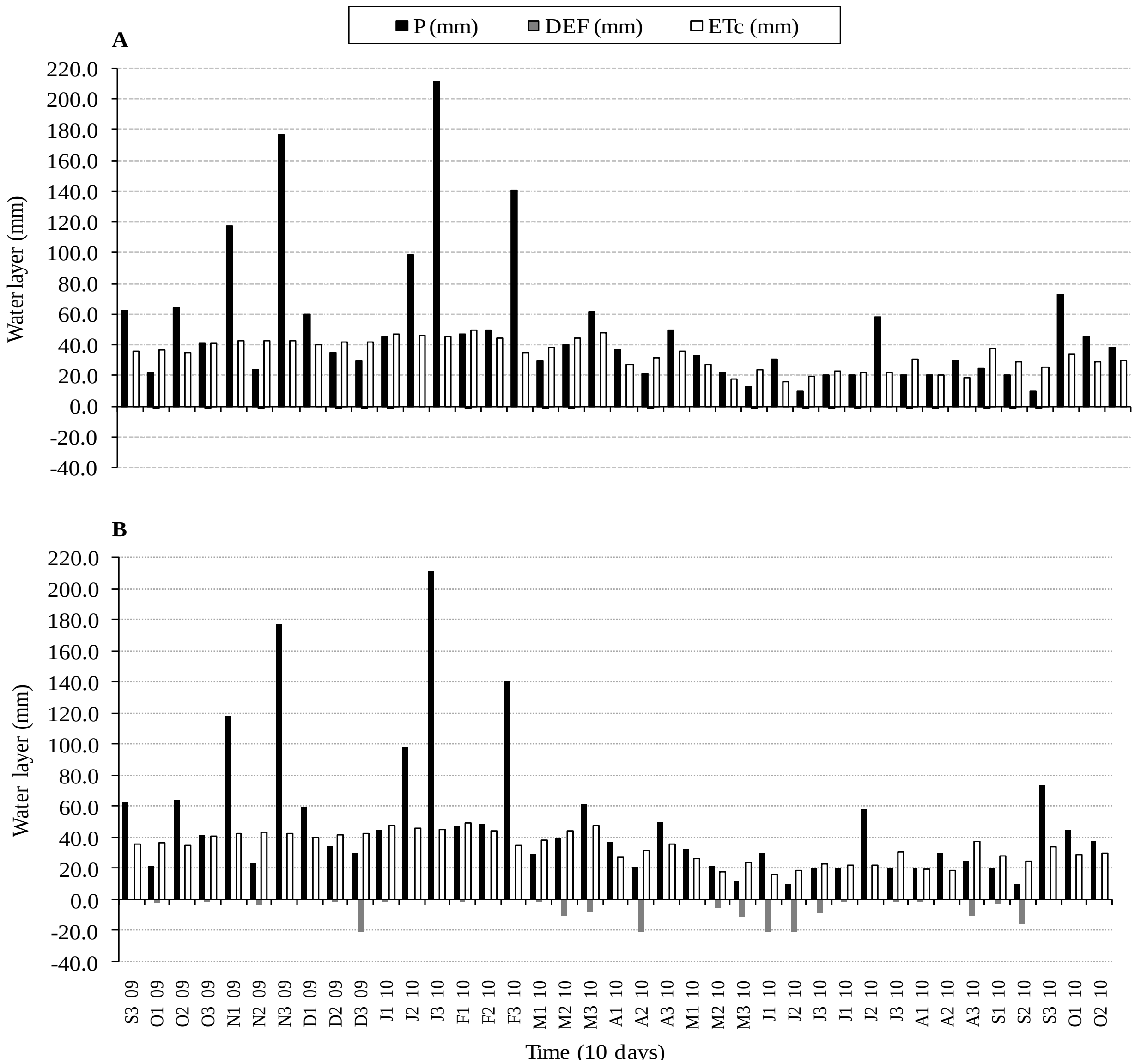

Fig. 1. Decendial water balance in subsurface drip by irrigation management (A) and non-irrigated management (B) from September/2009 to October/2010, Jaú, SP, Brazil. P = rainfall + water layer; DEF = water deficit; ETc = crop evapotranspiration**.**

Like other crops management practices including irrigation system significantly influence sugarcane crop (Sajjad et al., 2016). Subsurface drip irrigation system is among the most efficient irrigation system available for sugarcane cultivation. Although its initial cost is high, however, substantial amount of water is saved by reducing evaporative, runoff and drainage water losses (Camp, 1998; Barbosa et al., 2017). Drip irrigation has an additive advantage in the form of efficient nutrient application i.e., fertigation, contributing to the crop development (Bush et al., 2016). In the ratoon sugarcane crop potassium (K) and nitrogen (N) are the most applied nutrients in cover, where the amount of K varies from 150 to 300 kg ha^{-1} and N varies from 100 to 200 kg ha^{-1} (Vitti et al., 2015).

For maintaining higher crop yields N is the most extensively used fertilizer (Awan et al., 2016; Dar et al., 2016; Liu et al., 2015) and is the third largest nutrient extracted by sugarcane and its absorption occurs 99% via the mass flow (Oliveira et al., 2010). Nitrogen is an extremely important nutrient for crop vegetative growth, having direct influence on the crop yield, by the leaf area and plant height increase (Ata-Ul-Karim et al., 2016), due to the increase of photosynthetic rates, by the fact that N is present in CO_2 fixation enzymes acting as well as a structural component of the chlorophyll molecule.

The increase in sugarcane yield by irrigation is well documented (Wiedenfeld, 2004; Wiedenfeld and Enciso, 2008; Vieira et al., 2014; Silva et al., 2014; Oliveira et al., 2014). However, most of the sugarcane irrigation studies in Brazil used the central pivot system (Vieira et al., 2014), conventional sprinkler (Oliveira et al., 2014) and drip irrigation (Ferreira Júnior et al., 2014), having few studies that address the physiological parameters of sugarcane as a function of subsurface drip irrigation. Within this context, this study aimed to evaluate the effects of N fertilization applied via subsurface drip irrigation on physiological parameters and crop yield.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in the Experimental Station of the São Paulo Agency of Technology and Agribusiness, located close to Jaú, SP, Brazil (22°17'S and 48°34'W, 580 m altitude) from September 2009 to October 2010. According to the Köppen classification the region's climate is Aw, with tropical characteristics of rainy summers and dry winters, in which the driest month having precipitation less than 60 mm and less than 4% of the total annual precipitation. The air temperature average during the experimental period was 22.7° C. The soil was classified as Oxisol. Before the experiment installation, soil samples were collected from 0-20, 20-40 and 40-80 cm depth layers to carry out the chemical and grain size analysis (Table 1).

2.2 Crop management and experimental design

Sugarcane cultivar SP80-3280 was used during the third ratoon cycle. The experimental design adopted was the randomized blocks in a factorial 2×2 scheme, with four replications for each treatment. The variation factors were two water regime (irrigated and non-irrigated), and two levels of N fertilization (0 and 150 kg ha^{-1}). The aim of the treatment 0 kg ha^{-1} of N was that in periods of financial crisis some growers do not fertilize the sugarcane ratoon. The subsurface drip irrigation system was used for the irrigated treatment (Model DRIPNET PC 22135 FL, Adana, Turkey), with emitters spaced every 0.5 m and flow rate of 1.0 L h^{-1}, and the other treatment consisted of non-irrigated management (no irrigation). Urea (45% of N) was used as source of N. The experimental plots consisted of five lines of double furrow (0.40 m between simple rows in the double row x 1.40 m between the double rows) with 8.0 m in length, and between the rows dripper tubes were buried at 0.25 m deep.

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Table 1. Chemical and physics characterization of an eutrophic Argissolo soil (Jaú, SP, Brazil).

Depth	pH	O.M.	P resin	$S\text{-}SO_4$	K	Ca	Mg	H+Al	V
cm	$CaCl_2$	g dm^{-3}	mg dm^{-3}		$mmol_c dm^{-3}$				%
0-20	5.2	19	19	3.0	0.9	27	14	22	66
20-40	5.3	14	11	2.0	0.4	21	12	20	64
40-80	5.3	7.0	3.0	6.0	0.2	21	7.0	16	66

Depth	Sand	Silt	Clay	Textural class
cm	g kg^{-1}			
0-20	660	70	270	
20-40	600	60	340	Sandy clay
40-80	530	70	400	

O.M.: Organic Matter; V: Saturation Bases. Hydrometer methods (Bouyoucos, 1927).

The soil water condition was monitored during the experiment by pressure meters and irrigation frequency was calculated considering the maximum storage level or available water capacity of 70 mm of the soil, the rainfall, the atmospheric demand and sugarcane evapotranspiration. The total rainfall for the period was 1,435 mm and the amount of water applied by the irrigation system in the treatment was 392 mm, distributed during crop development cycle. Irrigation management was carried out taking into account the supply 100% of the crop's evapotranspiration, according to Penman-Monteith method (Howell et al., 2004), which had an accumulation of 1,320 mm (accumulated ETC). Then, a ten-day water balance estimation was performed (Fig.1) and the water stress was calculated (Fig.1B), which resulted in the amount of 519 mm

In the irrigated treatment, N was applied via subsurface drip irrigation twice a week, distributed between the previous harvest cycle until four months prior the final harvest, and 125.0 kg ha^{-1} of K in the form of potassium chloride (KCl) was also added. Fertilizer application ended three months before the final harvest. In the non-irrigated treatment all N and K were applied at October 2009, that is 30 days after the ratoon cut.

2.3 Physiological measurements

The determination of physiological parameters was carried out five times during the plant development, at 38, 121, 208, 291 and 381 days after the third cut (DAC), in one central meter of the double line of each plot.

2.4 Leaf area index

The leaf area index (LAI) was determined between 13:00 and 14:00 h with the use of a ceptometer (LP-80 mod., Decagon, WA, USA).

2.5 Estimation of the leaf chlorophyll content

Chlorophyll content (SPAD index) was estimated using a chlorophyll meter (mod. SPAD-502, Minolta Corp., Ramsey, NJ, USA). The average plot was composed by five readings taken from leaves +1 (top visible dewlap, TVD). The reading corresponds to leaf pigment content and its value is equivalent to the amount of light transmitted by the leaf in two wavelength regions, in which chlorophyll absorption is different (Malavolta et al., 1997). The reading value is, therefore, proportional to the amount of chlorophyll in the leaf.

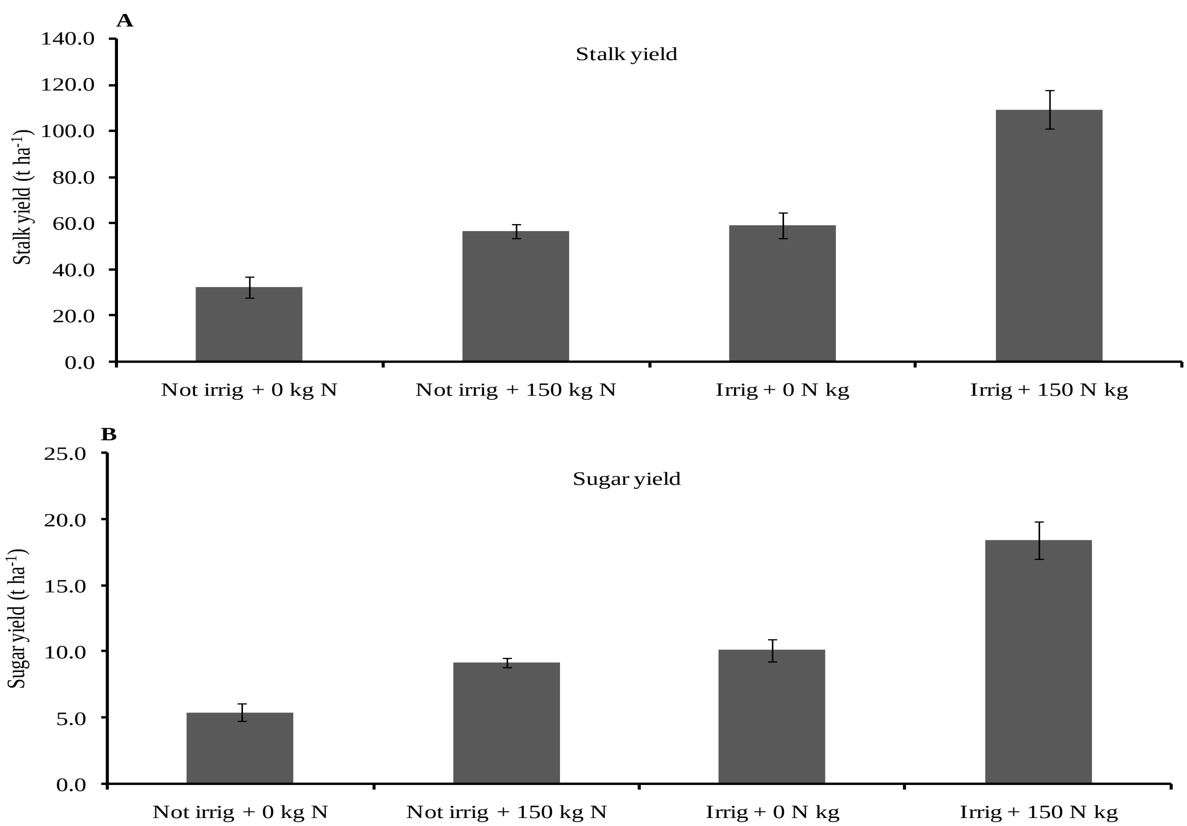


Fig. 2. Stalk (A) and sugar (B) yield of sugarcane under treatments not irrigated (Not irrig) and irrigated by subsurface drip (Irrig) with two levels of nitrogen, 0 and 150 kg of N ha^{-1}. (Each symbol indicates the average of four replicates ± standard deviation).

Table 2. Variance analysis (*F* values) of stalk and sucrose productivity in sugarcane, according to irrigation and nitrogen fertilization.

Variation Causes	Productivity of stalks	Productivity of sugar
Irrigation	193.82**	238.62**
Nitrogen	169.66**	180.19**
Irrigation × Nitrogen	20.76**	25.49**
C.V. (%)	8.85	8.35

** Significant at 5% (Tukey Test, p<0.05).

2.6 Total chlorophyll content

Chlorophyll content (*a*+*b*) was determined from ten discs removed from leaves +1 (TVD) of each portion and immersed in dimethyl formamide (DMF), and kept shielded from light for 24 hours. Afterwards, the absorbance reading was performed in a spectrophotometer at wavelengths of 647 and 664 nm; the reading was done on 1 mL of chlorophyll extract diluted in 1 ml of deionized water, in accordance to Porra et al. (1989).

2.7 Leaf relative water content

Leaf relative water content (RWC) was obtained in the laboratory by the extraction of two discs (0.69 cm^2 each) of the same leaf +1 and the fresh tissue mass (Wf) was determined in an analytical balance (Tecnal Equip. Lab., Piracicaba, SP, Brazil). The turgid tissue mass (Wt) was found after rehydration of the discs with deionized water for 24 h. The tissue dry mass (Wd) was found after the disks were dried for 48 hours at 80° C in an oven of forced air circulation. The RWC was calculated by formula, in accordance to the methodology presented by Jamaux et al. (1997).

2.8 Stomatal conductance

The stomatal conductance (g_s) was obtained by a porometer (Decagon Devices, Inc., Pullman, WA, USA) in the middle region of leaf +1, between 10:00 and 14:00 h.

2.9 Potential quantum efficiency of photosystem II

The potential quantum efficiency of photosystem II (F_v/F_m) in leaf +1 was found using a portable fluorometer (mod. OS-30p, Opti-Sciences, Inc., Hudson, NH, USA). The leaves were pre-darkened for 30 minutes with specific clips prior to the fluorescence measurements. The F_v/F_m variable was determined according to Maxwell and Johnson (2000), in which measurements were made between 10:00 and 14:00 h.

2.10 Stalk and sugar productivity

Stalk productivity (t stalks ha^{-1}, TCH) was determined by stalk weight at 381 DAC and the area occupied by each plot. The sugar productivity (t sugar ha^{-1}, TSH) was calculated by the product between TCH and the apparent sucrose percentage in stalks corresponding to each portion, divided by 100. To obtain the sucrose content, ten stalks were taken from each plot and analysed in the laboratory of the technological analysis in accordance to Tanimoto (1964).

2.11 Water use efficiency

Water use efficiency (WUE) was calculated in sugarcane crop on the basis of irrigation and N fertilization. The WUE was defined as the ratio between productivity and the total volume of water used in the production, considering only rainfall in rainfed treatment, and rainfall + irrigation in irrigated treatment, according to equations (1) and (2) (Farias et al., 2008):

$$WUE\text{stalk} = Pstalk/Wv \qquad [1]$$

Where WUE_{stalk} is (kg m^{-3}); Pstalk is productivity of stalk (kg ha^{-1}); Wv is total volume of water consumed (m^3 ha^{-1}).

$$WUE\text{sugar} = Psugar/Wv \qquad [2]$$

Where WUE_{sugar} is (kg m^{-3}); Sp is Productivity of sugar (kg sugar ha^{-1}); Wv is total volume of water consumed (m^3 ha^{-1}).

2.12 Data analysis

Data were subjected to the variance analysis by the F test followed by the Tukey test application (5%) for the comparison between averages.

3. Results

The TCH and TSH were significantly influenced by irrigation and N fertilization, as well as by a significant interaction occurring between these factors (Table 2). The highest TCH was found in the treatment with irrigation and application 150 kg N ha^{-1} along the crop cycle, which had 109.34 t ha^{-1} (Fig. 2A). The treatment with irrigation without addition of N obtained an average TCH of 59.2 t ha^{-1}. The lower TCHs were observed in treatments without supplemental irrigation with N, 56.6 t ha^{-1}, and without N, 32.0 t ha^{-1}. Similar response pattern was observed in TSH (Fig. 2B). The highest TSH was obtained in the treatment with irrigation with N (8.47 t ha^{-1}) and the lowest in the treatment without irrigation and without N (5.4 t ha^{-1}) (Fig. 2B).

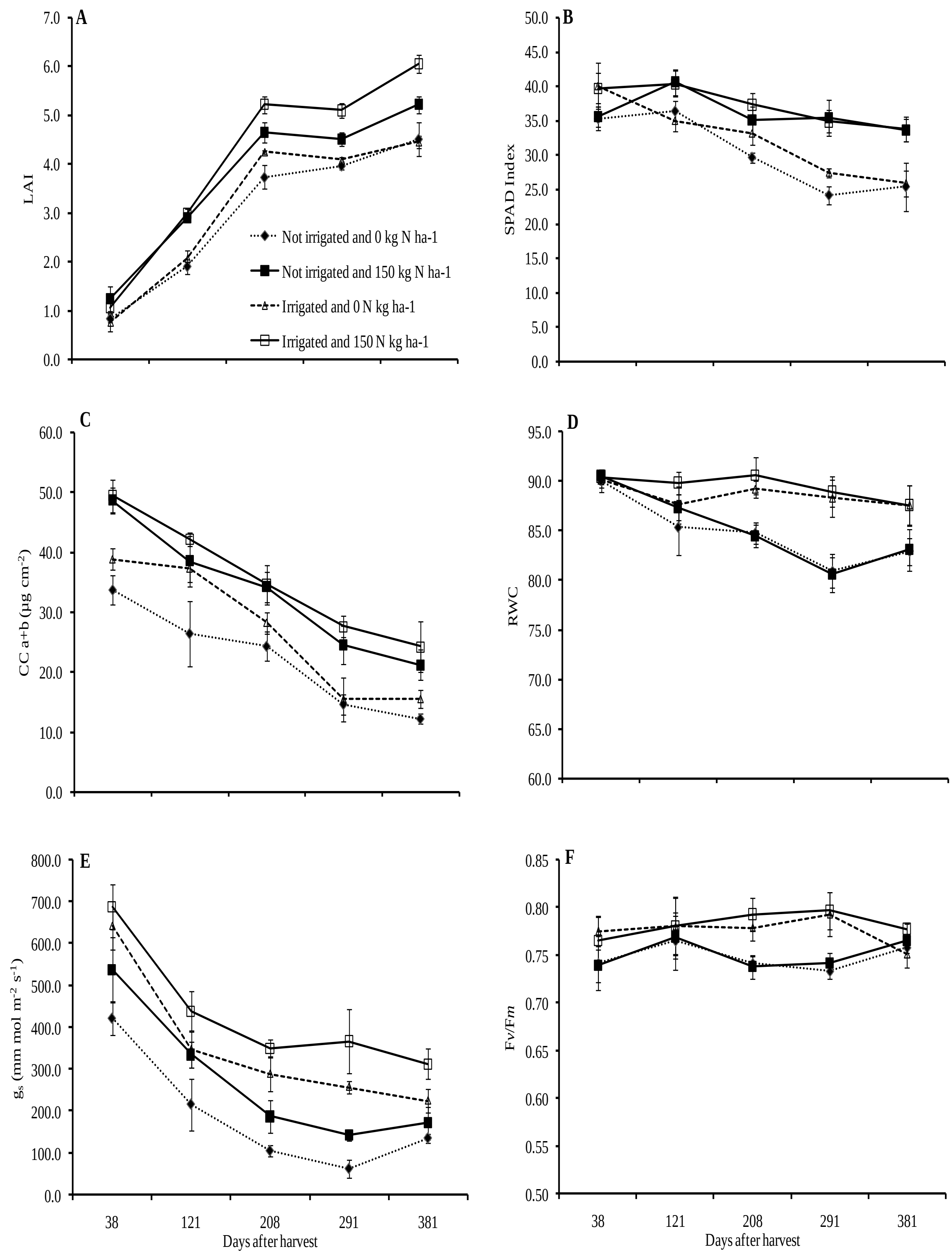


Fig. 3. Physiological parameters of sugarcane, leaf area index (LAI) (A); SPAD index (B); chlorophyll content *a+b* (Chl) (C); water relative content (RWC) (D); Stomatal conductance (g_s) (E); potential quantum efficiency of photosystem II (F_v/F_m) (F), under treatments not irrigated and irrigated by subsurface drip irrigation with two levels of nitrogen, 0 and 150 kg of N ha^{-1}. (Each symbol indicates the average of four replicates ± standard deviation).

Table 3. Variance analysis (*F* values) of physiological parameters of sugarcane crop under drip irrigation and nitrogen fertilization at five evaluation periods.

Variation Causes	F_v/F_m	SPAD	LAI	g_s	RWC	Chl *a+b*
38 days after harvest of previous cycle						
Irrigation	9.77**	21.73**	1.17ns	20.56**	0.09ns	3.04ns
Nitrogen	0.38ns	0.00ns	13.04**	5.27*	0.91ns	134**
Irrigation × Nitrogen	0.32ns	0.34ns	0.75ns	5.04*	0.07ns	9.46**
C.V. (%)	2.78	5.44	19.93	12.27	0.92	5.15
121 days after harvest						
Irrigation	0.89ns	0.97ns	4.77*	25.14**	6.54*	15.80**
Nitrogen	0.01ns	31.16**	245**	20.12**	5.08*	21.41**
Irrigation × Nitrogen	0.01ns	0.44ns	0.28ns	0.35ns	0.01ns	3.93ns
C.V. (%)	3.65	4.52	4.92	14.00	2.13	10.12
208 days after harvest						
Irrigation	49.51**	14.94**	36.59**	122.11**	64.22**	2.87ns
Nitrogen	0.67ns	42.68**	108.06**	20.84**	0.61ns	39.57**
Irrigation × Nitrogen	1.73ns	0.63ns	0.069ns	0.42ns	1.65ns	1.83*
C.V. (%)	1.72	4,41	4,05	13,5	1,5	8,35
291 days after harvest						
Irrigation	4.46ns	2.72ns	37.36**	103.2**	82.36**	2.13ns
Nitrogen	13.91**	123.6**	185.5**	21.12**	0.02ns	65.42**
Irrigation × Nitrogen	10.76**	4.95*	16.49**	0.51ns	0.34ns	0.71ns
C.V. (%)	2.21	5.55	2.60	19.94	2.04	13.25
381 days after harvest						
Irrigation	0.19ns	0.06ns	12.21**	55.49**	23.34**	5.85*
Nitrogen	7.29*	46.44**	107.0**	16.01**	0.00ns	46.08**
Irrigation × Nitrogen	2.34ns	0.02ns	16.10**	2.85ns	0.01ns	0.00ns
C.V. (%)	1.63	7.98	4.39	14.69	2.22	14.32

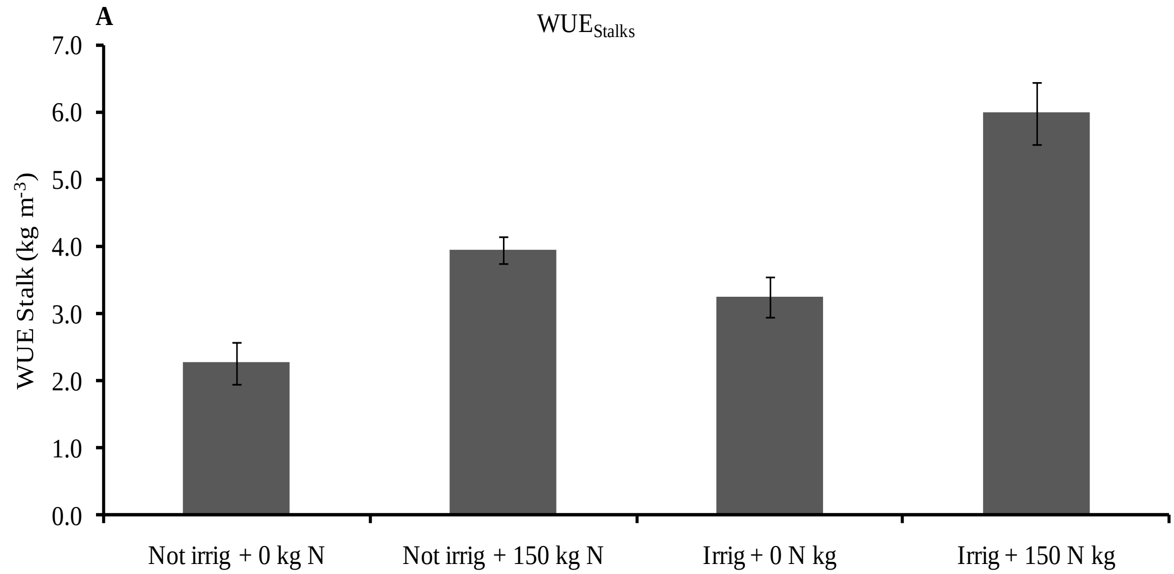
Potential quantum efficiency of photosystem II (F_v/F_m); SPAD index; leaf area index (LAI); Stomatal conductance (g_s); water relative content (RWC); Chlorophyll content *a+b* (Chl);

Regarding the physiological parameters, irrigation and N fertilization promoted positive effects during the crop development cycle (Table 3). The leaf area index (LAI) was increased in all treatments during sugarcane development (Fig. 3A), however the LAI at harvest was higher in the treatment with irrigation and with N fertilization (6.0), followed by the treatment without irrigation plus N fertilization (5.2). The lower LAI was observed in the treatment with no irrigation and no N fertilization (4.5).

SPAD index was decreased along the cycle progress (Fig. 3B). It was also observed that there was a greater effect of N fertilization than irrigation on this parameter, in which the highest values of SPAD index were observed in plants that received the N fertilizer, specifically in the 208-381 DAC period. At 208 DAC, the average SPAD index in the treatments with N was 35.1 and in the treatments without N were 25.8.

At 381 DAC, the average SPAD index was 33.7 for the treatments with N and 25.6 for those without N. As well as SPAD index, the chlorophyll content *a* + *b* (Chl) decreased over time (Fig. 3C), and it was higher in N fertilization treatments, throughout all the experimental period.

Irrigation maintained relative water content (RWC) of sugarcane plants (Fig. 3D). It was observed that RWC of irrigated plants were always superior to 87% at 208 days after harvest, unlike the treatments without irrigation, and that at 291 days after harvest it reached values of 80%. There was no effect of N fertilization on the RWC in any crop growth period.



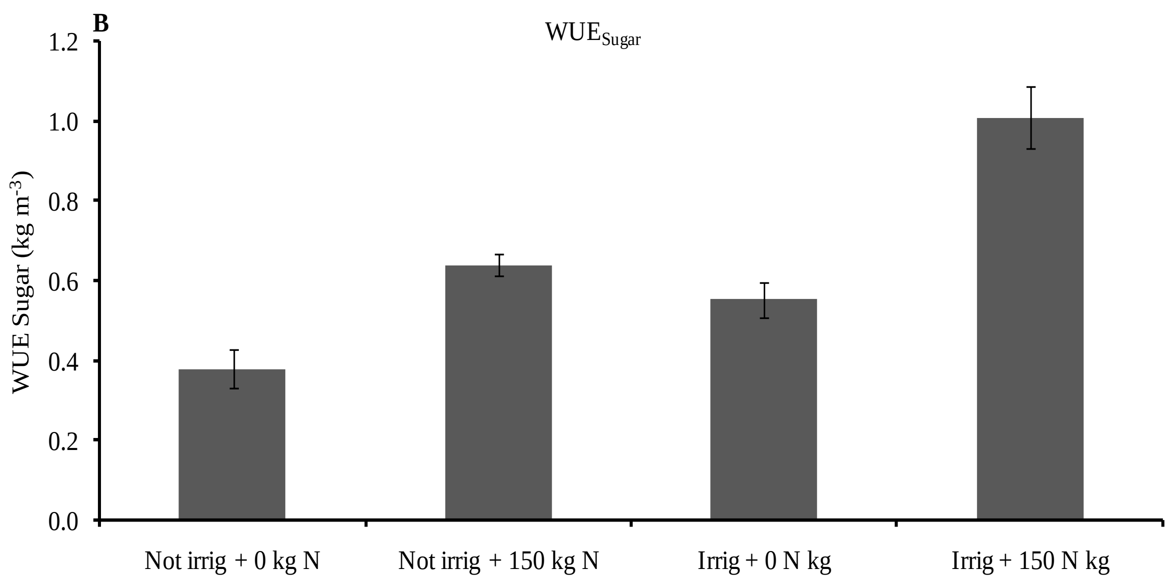


Fig. 4. Water use efficiency for sugarcane stalk (WUE$_{stalk}$) (A) and sugar (WUE$_{sugar}$) (B) under treatments without (Not irrig) and with irrigation (Irrig) with two nitrogen levels, 0 and 150 kg of N ha^{-1}. (Each symbol indicates the average of four replicates ± standard deviation).

The reduction of the water level in the soil decreased the stomatal conductance (g_s) in all treatments over time (Fig. 3E). However, it was observed that treatments receiving irrigation always had higher g_s than the non-irrigated treatments.

Between 208 and 381 DAC the g_s in the treatment with irrigation and N fertilization was 343.6 mmol m^{-2} s^{-1}, followed by the treatment with irrigation and without N (257 mmol m^{-2} s^{-1}), the lowest values were observed in the treatments with no irrigation, i.e., in the non-irrigated with N the g_s was 166.9 mmol m^{-2} s^{-1}, and for the non-irrigated without N g_s was 100.9 mmol m^{-2} s^{-1}. Therefore, it was noticed that highest values for g_s were obtained in the irrigated treatments, and the N fertilizer contributed to the g_s increase within each water regime.

There was a decrease in the potential quantum efficiency of photosystem II (F_v/F_m) during the sugarcane grand growth stage (208 and 291 DAC) in non-irrigated treatments (Fig. 3F). Since at 291 and 208 DAC the F_v/F_m values of irrigated treatments were close to 0.78 - 0.80, while in the non-irrigated treatments F_v/F_m values were close to 0.73 - 0.74. At

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381 DAC there was no difference in F_v/F_m among treatments.

Water use efficiency for stalk production (WUE_{stalk}) was strongly influenced by the treatments (Table 1; Fig 4A). The lowest WUE_{stalk} was found in the treatment with no irrigation and without N fertilization (2.26 kg m^{-3}), while the WUE_{stalk} was 3.94 kg m^{-3} in the treatment without irrigation and with N fertilizer, that is, an increase of 74%. The WUE_{stalk} in the treatment with irrigation and without N was 3.24 kg m^{-3}, while with N, the WUE_{stalk} was 5.99 kg m^{-3}, representing an increase of 84%.

It has been observed that the WUE_{stalk} in the treatment with no irrigation and with N was 21% greater than the WUE_{stalk} of treatment with irrigation and N. As well as observed in WUE_{stalk}, the highest WUE_{sugar} was obtained in the treatment with irrigation and with N (1.0 kg m^{-3}), followed by treatments without irrigation and with N (0.63 kg m^{-3}), with irrigation and N (0.55 kg m^{-3}), and non-irrigated without N (0.37 kg m^{-3}).

4. Discussion

It was observed that both irrigation and N fertilization promoted positive increases in sugarcane stalk productivity. Irrigation in sugarcane crop promoted an increase of 82.15% in TCH in the treatment without N fertilization, and an increase of 92.94% in the treatment with N fertilization (Fig. 2A). Nitrogen fertilization promoted an increase of 74.36% in TCH in the treatment without irrigation and an increase of 84.64% in irrigated treatment. Therefore, water supply increased the efficiency of N fertilization in sugarcane culture, which in turn, contributed to increase stalk and sugar productivity.

The positive effects of water on sugarcane development have been reported by several authors. Well watered sugarcane plants have better development of shoots (leaf area, number of green leaves, leaf length and width), higher development of the root system and mainly higher stalk and sugar yield (Inman-Bamber and Smith, 2005; Barbosa et al., 2015; Koonjah et al., 2016). However, all sugarcane produced in the São Paulo state, Brazil, is on non-irrigated areas, therefore, this study highlights irrigation importance as a technique to increase sugar and stalk productivity of sugarcane.

Other studies already demonstrated the positive results of irrigation on crop yield. Oliveira et al. (2011) studied the effects of irrigation on eleven sugarcane cultivars and observed an average increase of 145% in stalk and 151% in sugar productivity. Vieira et al. (2014) also reported a productivity increase on sugarcane between 70.0 and 150.0% according to the different irrigation levels.

Nevertheless, our results showed that stalks and sugar productivity were increased with N supplementation in irrigated sugarcane crop. These results are in agreement with Wiedenfeld (1995), on studies carried out in sugarcane ratoon in Texas (USA), in which the best results of N were observed in treatments with higher levels of irrigation, while at low water levels N response was not so pronounced, since if water is the limiting factor, the crop doesn't take advantage of the increased N availability. Wiedenfeld and Enciso (2008) reported a linear increase in sugarcane productivity in response to N fertilization, however, for that increase to occur, the soil moisture has to have ideal conditions for crop growth.

In general, in all treatments throughout the experimental period it was observed that there was LAI increase, and reduction of SPAD index, Chl, and g_s (Fig. 3). Regarding RWC and F_v/F_m did not show large variations depending on the crop development, but the differences obtained were according to the treatments applied. The LAI increase in sugarcane according to crop development is already well known through various researchs. As well as the reduction for SPAD index, Chl and g_s occur due to the crop physiological aging process (Rhein and Silva, 2017).

Irrigation and N fertilization also influenced the plant physiological parameters (Fig. 3). Regarding SPAD index, LAI and Chl was observed that the higher values were obtained in the treatments with N, both with irrigation or not. Studies have shown a positive correlation between SPAD index and the chlorophyll content in conditions of water deficit or not. Zhao et al. (2014) studied the effect of four levels of N (0, 75, 150 and 225 kg ha^{-1}) on sugarcane crop and observed a linear increase of the leaf area according to the N fertilization, as well as an increase in SPAD index in the leaves. Therefore, the increase of SPAD index, Chl and LAI due to N fertilization is related to the fact that N is the main component of chlorophyll and enzymes assimilation of CO_2, phosphoenolpyruvate (PEP) and ribulose bisphosphate carboxylase/oxygenase (Rubisco), and that this nutrients' presence heavily contributes to the leaf area increase in sugarcane (Robinson et al., 2013).

The treatment without irrigation and with N fertilization resulted in higher values of SPAD index, Chl and LAI than the treatment with irrigation and

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without N (Fig. 3), however, TCH was lower in the treatment without irrigation and with N than the irrigated treatment (Fig. 2A). Two factors may have influenced this response, the first one referring to the fact that C4 species require less N, due to the low protein content in the mesophyllic chloroplasts, but also 50% of leaf N is present in Rubisco, therefore, C4 species are less demanding in N because of the greater efficiency of carbon carboxylation, consequently, high concentrations of N stimulate the plant vegetative growth, specifically the leaf area growth. The second factor relates to the lower RWC on non-irrigated plants, since in conditions of low water content in the leaf tissue the photosynthesis may be limited due to the stomatal closure (g_s) and also the plants photochemical efficiency reduction (F_v/F_m). Therefore, despite N fertilization increasing the plants Chl and SPAD index, crop development was limited by the low water availability.

Between 208 and 291 DAC, the period with higher water restriction to the crop development (Fig. 1B), irrigation rendered positive effects on RWC, g_s and F_v/F_m of plants, however, no effect of N fertilization was observed (Fig. 3). The RWC is an indicator often used to measure the plant water status (Welbaum, 2013), therefore, it was noticed that from 208 DAC, the RWC of non-irrigated plants decreased to values approaching 80%, while irrigated plants had higher RWC (Fig. 3D), contributing to the fact that metabolic processes were not strongly affected by water deficiency. Silva et al. (2014), in a study with 78 genotypes of sugarcane, observed that the average RWC of irrigated plants was 89%, since the RWC of non-irrigated plants was 80% and positive relationship between RWC and TCH could also be observed, specifically in genotypes susceptible to drought. Other studies already reported a RWC reduction in sugarcane under drought (Silva et al., 2007; Sato et al., 2010; Graça et al., 2010).

Beyond reducing RWC, a higher reduction of g_s in non-irrigated plants was also observed (Fig. 3E). Barbosa et al. (2015) observed that water deficit reduced the g_s of sugarcane leaf in three developmental stages (young-1, adult+1 and senescent), as well as a reduction in the leaf carbon daily balance in those conditions. Other studies also showed the g_s reduction in treatments with water deficit or lower level of water in the soil (Machado et al., 2009; Gonçalves et al., 2010; Zhao et al., 2013; Eksteen et al., 2014).

The g_s reduction is strongly correlated with RWC reduction, as the plant controls RWC through the stomatal opening and closure, having g_s reduction as one of the first plant strategies to prevent the leaves excessive dehydration (Inman-Bamber and Smith, 2005). Besides the RWC and g_s reductions in non-irrigated plants, a reduction of the potential quantum efficiency of photosystem II (F_v/F_m) was also observed, specifically in the period between 208 and 291 DAC (Fig. 3F). The F_v/F_m of irrigated plants ranged from 0.78 to 0.80, while the F_v/F_m of non-irrigated plants ranged from 0.73 to 0.74.

In a study conducted by Silva et al. (2011), sugarcane genotypes with F_v/F_m values below 0.76 already showed signs of stress due to water deficit. Therefore, in the period from 208 to 291 DAC non-irrigated plants showed photo inhibition signs of photosystem II due to reduced F_v/F_m, while at 381 DAC, period with an increased amount of rain (Fig. 1), the non-irrigated plants F_v/F_m was close to irrigated plants values.

The F_v/F_m reduction of sugarcane plants is positively correlated with the reduction of TCH (Silva et al., 2012). Ability of plants to maintain high values of F_v/F_m under drought conditions is characteristic of tolerant plants (Silva et al., 2007). According to Pimentel (2014), the lack of water induces the stomatal closure and reduces the CO_2 availability, causing energy excess, which in O_2 presence can cause damage to photosystem II, particularly in the thylakoids membrane of chloroplasts, as a result, there is a reduction of photosynthesis and the plant growth. Therefore, the present results demonstrate that the F_v/F_m reduction of non-irrigated plants during the period of increased drought contributed to decrease TCH. And, it was also observed that the SP80-3280 cultivar is not tolerant to water deficit, requiring good soil moisture to grow.

There was great effect of irrigation and N fertilization on water use efficiency for stalk production (WUE_{stalk}) (Fig. 4A). It can be noted that fertilization with N somewhat compensated the lack of irrigation, since in the treatment without irrigation and with N WUE_{stalk} was higher than in the treatment with irrigation and without N, a corresponding increase of 21%, as well as, irrigation increased N fertilization efficiency, since in the treatment with irrigation and without N, the WUE_{stalk} was 3.24 kg m^{-3}, while in the treatment with irrigation and with N the WUE_{stalk} was 5.99 kg m^{-3}, a increase of 84%. Therefore, our results demonstrated that N fertilization efficiency is increased by irrigation, in the same way that irrigation has greater efficiency in the presence of N.

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Doorenbos and Kassam (1979), reported that WUE in sugarcane culture can vary 5-8 kg m^{-3}. About treatments evaluated in this study, only with irrigation and with N reached that values, therefore, the WUE_{stalk} of sugarcane in drought regions is below the optimal values for the crop, which can be caused both by reduced water availability and by the large reduction in crop yield in this agricultural system. Similar results were observed by Farias et al. (2008), when under non-irrigated treatment the WUE_{stalk} was 2.99 kg m^{-3}, however when irrigation was used corresponding to 25% of evapotranspiration, the WUE_{stalk} increased to 5.31 kg m^{-3}, and reaching 7.31 at 50% of crop evapotranspiration. In a study performed by Oliveira et al. (2011) with eleven sugarcane cultivars, it was observed that irrigation increased WUE about 99% compared to the treatment performed in non-irrigated management.

The WUE_{sugar} had similar response to WUE_{stalk} (Fig. 4B), this occurred because sucrose is stored in stalks, therefore, the higher stalk yield per hectare, the greater sucrose production per hectare. Response observed as well by Farias et al. (2008). Therefore, WUE increased with irrigation by the fact that the performance gain percentage is greater than surplus supplied water percentage. In this research could be observed that the 27% increase in irrigation (390 mm of water) generated up to 84 and 94% in stalk yield in treatments with and without N, respectively. Farias et al. (2008) observed that with an increase of 28% in the water application the stalk yield increased by 132%. Likewise Oliveira et al. (2011), with increase of 22% in the irrigation obtained an average increase of 145% in stalk yield.

Therefore, the use of irrigation in sugarcane during drought periods and the phase of grand crop growth increases TCH and TSH, as well as WUE, and yet, as our results showed, these increases are more significant when irrigation is combined with the application of N, creating a win-win, that is, irrigation increasing N fertilizer efficiency and N fertilization increasing irrigation efficiency, as observed by Wiedenfeld (2000) and Otto et al., 2016..

Regarding the irrigation system, our results showed that the subsurface drip irrigation in sugarcane crop had positive results, generating an increase on crop yield. Xu et al. (2010) also observed positive effects of the subsurface drip irrigation combined with nutrients application in sugarcane. In more recent studies, Oliveira et al. (2014) also observed a 40% increase in stalk yield with the subsurface drip irrigation use, as well as positive effects of N application along with irrigation. Better water use for sugarcane cultivation by subsurface drip fertigation was also reported by Scarpare et al., (2016).

5. Conclusion

The effects of irrigation and nitrogen fertilization on yield and on physiological parameters of sugarcane crops are positive. Our results showed that stalk production of sugarcane increased with irrigation, and that nitrogen fertilization contributed even more to this increase. It is also observed that at the time of greatest water deficit for crop, the plants of non-irrigated treatments had RWC, g_s and F_v/F_m reduced, and as a result there was a yield reduction of stalks and sugar. Nitrogen fertilization also proved to be an important strategy to increase crop yield, and the highest stalk and sugar yield occurred in the treatment with irrigation and with nitrogen. The fertilization with nitrogen also contributed to the LAI, SPAD index and Chl increase, however, our results showed that the nitrogen fertilizer has its efficiency increased with irrigation. Therefore, the surface drip irrigation with nitrogen fertilization is an excellent tool for increasing crop yield and WUE, as well as an important tool to enhance the effects caused by drought in sugarcane crop.

List of abbreviations: Chl, Chlorophyll; K, Potassium; N, Nitrogen; SPAD index, Chlorophyll content; WUE, water use efficiency; KCl, potassium chloride; DAC, days after the third cut; LAI, leaf area index; TVD, top visible dewlap; RWC, relative water content; g_s, stomatal conductance; F_v/F_m, potential quantum efficiency of photosystem II; TCH, stalk productivity; TSH, sugar productivity.

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Author Contribution: MAS planned and coordinated the research, participated in the analysis and interpretation of data; AFLR: set up and carried out the field experiment, collected data; AMB:

performed analysis and data interpretation. MAS, AFLR and AMB wrote the article.

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