Physiological Assessments of Sweet Sorghum Inoculated with Azospirillumbrasilense according to Nitrogen Fertilization and Plant Growth Regulators

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Abstract—*Some factors, such as yield increase and production cost reduction, must still be assessed as a way toimprove the sweet sorghum success prospects. The use of plant growth regulator mixtures has shown significant results in crop yield.Nitrogen assimilation stands out as one of the main limiting factors in plant production. Given the physiological effects of plant growth regulators and their mode of action in the photosynthetic metabolism, the aim of the current study is to assess the physiological responses of sweet sorghum plants inoculated with Azospirillum brasilenseto the use of nitrogen fertilization and plant growth regulators. The experiment comprised split plots, with four repetitions. The treatments comprised nitrogen (0, 40, 80, 120 and 160 kg ha^{-1}) and biostimulant levels (0, 300, 400, 500 and 600 mL ha^{-1}) in sweet sorghum culture inoculated with Azospirillum brasilense. Variables such as gas exchange and total recoverable sugars (TRS%) were assessed. The application of plant growth regulator at the dose 440.7 ml ha-1 showed the highest photosynthetic efficiency up to 46 DAE. According to the experimental conditions, the plant growth regulator treatment had no effect on the TRS %. It is concluded that the plant growth regulator did not affect the production of sugars by the plant.*

Keywords— total sugars, kinetin, photosynthesis, inoculation

I. INTRODUCTION

Sweet sorghum (Sorghum bicolor Moench) is the name given to the sorghum plant able to produce and accumulate sucrose in the stem, reaching amounts close to those of the sugarcane.

The sorghum belongs to the family Poaceae. It is believed that its center of origin lies on the region where Sudan and Ethiopia are located in the African continent and that; apparently, it was disseminated worldwide by the native Africans who were smuggled into slavery. Nowadays, it is grown in most tropical and subtropical regions and is the fifth most produced cereal worldwide (MOREIRA, 2011).

Sorghum is considered a positive energy balance culture and stands out as a promising raw material to generate bioenergy, due to its high fermentable fiber and sugar contents, which may be exploited on a large scale and with great adaptability to different weather and soil conditions (SANTOS et al., 2014).In addition, the short production cycle (six months for high biomass yield) and the low water demand, in comparison to that of the sugarcane and corn, favor the sorghum, which shows better energy efficiency (NAGAIAH et al., 2012; SERNA-SALDÍVAR et al., 2012).

In addition to the sucrose production by sorghum, it can be said that some factors, such as productivity increase and crop production cost reduction, are still to be assessed as a way to improve the sweet sorghum success prospects. Among such factors, the high cost of fertilizers is a bottleneck that narrows the cost/benefit ratio in the sweet sorghum cultivation and in the sugar and alcohol generation (MOURA et al., 2005).

Although nitrogen is abundant in the atmosphere, the plants metabolize it just in its nitric and ammonia forms. Nitrogen fertilizers are expensive, and the biosphere reserve comes from the atmosphere.It cannot be directly assimilated by plants in its molecular form; however, the biological N fixation processes by prokaryotic organisms make such assimilation possible. The proliferation of soil bacteria adhered to the plant root surface was discovered in the early nineteenth century, along with the discovery of nitrogen fixation (MOREIRA, 2007).

Thus, the use of nitrogen-fixing bacteria benefited the yield of several crops such as soybean (Glicine max (L.) Merrill). Therefore, whenever nitrogen-fixing bacteria are inoculated in seeds, there is potential release nitrogen of approximately 150 kg N ha^{-1}throughout the crop cycle. Another advantage is the nutrient availability according to the needs of each stage of the cycle, which increases the plant metabolic efficiency and productivity (ARAÚJO; HUNGRIA, 1999).

Plant growth regulators are substances or a mixture of substances that may be directly applied to the plants in order to enable changes in the vital and structural processes, as well as to allow increasing the sucrose levels, the maturation time and the productivity (CASILLAS et al., 1986).

It is known that the sweet sorghum crop has been gaining prominence in the sucrose production chain, despite the high consumption of nitrogen fertilizers, and that the Azospirilum brasilense bacteria provides low-cost nitrogen, and even that the use of plant growth regulators is able to improve plant metabolism. Therefore, it is necessary assessing the effects of the inoculation with Azospirillum brasilense and of different doses of nitrogen fertilizer, as well as of the plant growth regulator Stimulate®, on the plant photosynthesis and on the total recoverable sugar production. It is also necessary assessing the relation between the plant growth regulator doses at different nitrogen fertilization levels and the behavior of phenological features such as the leaf area index and the dry mass weight of plants.

Thus, the hypothesis of the current study is that the physiological action of the plant growth regulator Stimulate®-based product, in interaction with the nitrogen fertilization, affects the photosynthetic metabolism by increasing the total recoverable sugar production in sweet sorghum plants inoculated with Azospirillum brasilense.

The aim of the current study is to assess the physiological responses of sweet sorghum plants inoculated with Azospirillum brasilense to nitrogen fertilization and to plant growth regulators.

II. MATERIAL AND METHOD

The experiment was conducted at Lagoa da Cruz Farm, which belongs to the Catholic University Dom Bosco, located in Campo Grande County– Mato Grosso do Sul State (20º23'16.59" S; 54º36'44.47" W; altitude 642 m).

The experiment comprised split plots, which were distributed in a randomized block design with four repetitions. The main plots encompassed 11 plant rows, with spacing of 0.50 cm and 0.35 cm between the plants in the row. The subplots corresponded to a row in the main plot alternated by an untreated border row. The treatments comprised the nitrogen levels (0, 40, 80, 120 and 160 kg ha^{-1}) in the main plots and the plant growth regulator Stimulate®levels (0, 300, 400, 500 and 600 mL ha-1) in the subplots.

The BRS 505 sweet sorghum cultivar of Embrapa, inoculated with Azospirillum brasilense, was used in the herein performed experiment.The Azospirillum brasilense bacteria strains Abv5 and Abv6 were used at the concentration of 2.108 bacteria per mL. The bacterial solution was applied to the seeds that showed no chemical treatment in the experiment sowing day. The treatments with different nitrogen levels were performed at 10 DAE (days after emergence) -when the plants started developing their secondary root system - using urea as N vehicle (45% nitrogen); the urea was calculated according to the area of each plot, and applied and incorporated to the soil.

The treatment with different plant growth regulator levels used the commercial product Stimulate®, which is manufactured by Stoller do Brasil. Its composition comprised the mixture of 0.009% cytokinein (*N6-furfuryladenine*, kinetin (Kt)), 0.005% auxin (4-Indole-3-butyric *acid* (IBA)) and 0.005% gibberellin(gibberellic acid (GA 3)).

The plant growth regulator mixture was applied through leaf spraying, using a boom sprayer with constant CO_2 pressure and flat fan type nozzle (110-02) at 15 DAE, when the culture showed secondary root system development.

The culture was conducted until 90 DAE, which was the physiological maturation period of the stems, when the culture presented more than 50% open panicles.

The gas exchange assessments were performed at 16, 46 and 81 DAE (period that corresponded to the beginning of the culture physiological maturation), using the Infrared Gas Analyzer - IRGA equipment (LI-6400 model, Li-Cor).

The technological parameters of sugar - POL%, RS% and TRS% - were determined according to the methodology by the Board of Sugarcane, Sugar and Alcohol Producers of São Paulo State (CONSECANA, 2001).

III. RESULTS AND DISCUSSION

Figure 2 presents the effect of the treatment with different nitrogen doses on the carbon assimilation in the plots that were not subjected to the application of plant growth regulators. The assessments performed at 16 DAE found that this parameter showed maximum response at the dose of 63.64 kg ha^{-1} N and reached 37.8 µmol m^2 s^{-1} of CO_2 assimilation.
The carboxilative assimilation increased as the N levels increased. The carboxylation decreased at the dose of 65.7 kg ha^{-1} N, at 81 DAE, and it may be a prerequisite to the N synthesis decrease. The carboxylation increase due to the increased nitrogen availability results from the need of carbon chains in order to assimilate nitrate (SOARES et al., 2013).

According to Malavolta et al. (1999), the plant production may always increase whenever each growth factor is present in the minimum quantity rather than in the optimum quantity. Thus, according to the findings of the aforementioned study, at 16 DAE, which is the period during which the sweet sorghum crop is in the early development stages and the apical and root meristems are growing, there is the optimum nitrogen quantity that may be considered ideal for such period, in addition to the need of CO_2in the photosynthetic metabolism. However, from the 46 DAE on, as long as the crop is growing and developing, and thus requiring higher carbon and nitrogen amounts, the increased nitrogen availability increases the carboxylation (Figure 1).

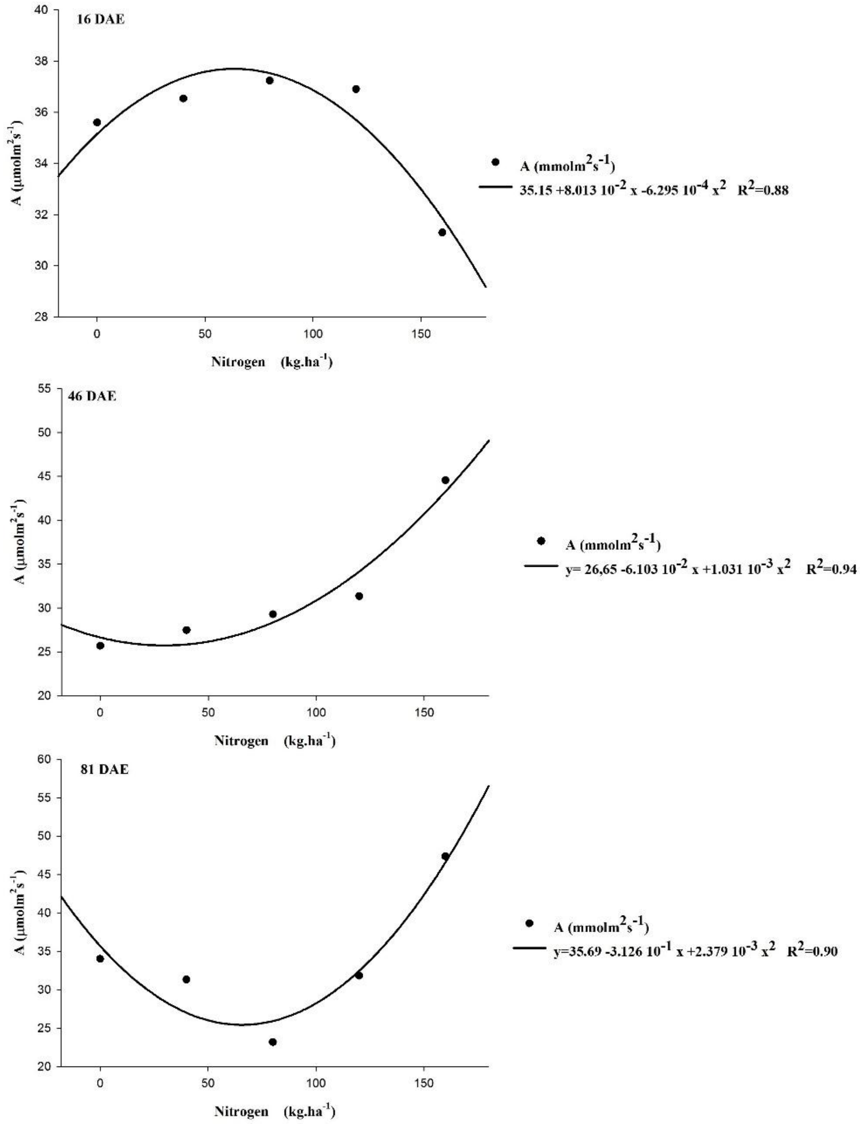

FIGURE 1. THE EFFECT OF NITROGEN CONCENTRATIONS (0, 40, 80, 120 AND 160 KG HA^{-1}) ON THE CO_2ASSIMILATION (A, µMOLM^{2}S^{-1}) AT 16,46 AND 81DAE IN SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE (*P≤0.05)

Figure 2 presents the results of carbon assimilation in plants inoculated with Azospirillum brasilense and treated with the plant growth regulator in all the three herein assessed periods. The assessments performed at 16 DAE showed that the plant growth regulator concentration increase led to linear increase in the momentary CO_2 assimilation in plants that were not subjected to nitrogen fertilization. This result may be considered significant, since the carboxylation increased due to the increased plant growth regulator doses.

As the culture developed, the plant growth regulator showed higher carboxylation at 46 DAE, at the dose of 424.9 mL ha^{-1} without nitrogen fertilization. No significant action of the plant growth regulator was observed under the same conditions at 81 DAE. The plants inoculated with plant growth regulator also showed increased carboxylation at 16 DAE.

In view of the positive action between the plant growth regulator and the inoculation, Fagan et al. (2015) stated that the cytokinins increase the gene expression for nodulation and enable the bacterial infection process in the roots. The authors also reported that the auxins stimulate the division of pericycle cells, thus increasing the proportion of lateral roots, but theyobey the maximum concentration required by the organ.

The cytokinins control the nitrogen absorption (MARTIN et al., 2000; FAGAN et al., 2015) and indicate the availability status of the nitrogen suitable to the plant by inhibiting the nitrate absorption system in the roots (FAGAN et al., 2015).

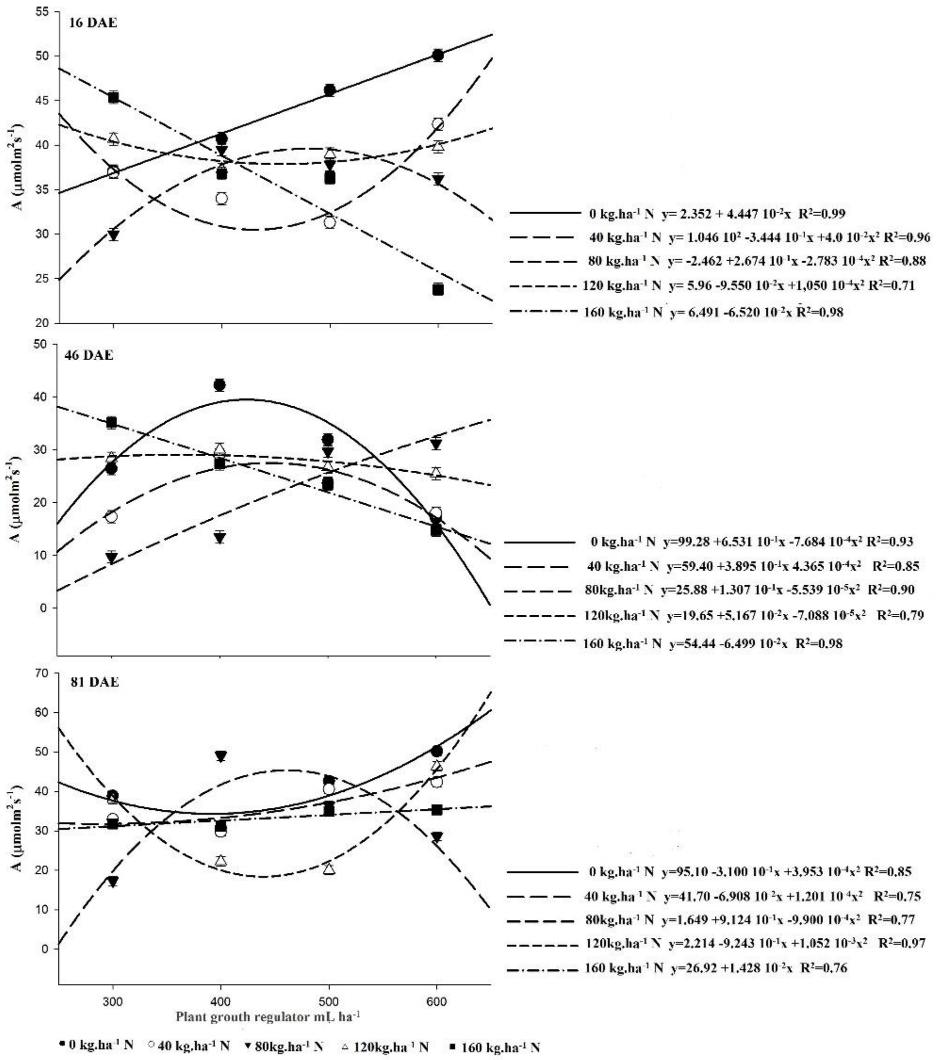

FIGURE 2.THE EFFECT OF THE PLANT GROWTH REGULATOR CONCENTRATIONS (300,400,500 AND 600ML HA^{-1}) ON THE CO_2 ASSIMILATION (A, µMOLM^{2}S^{-1}) AT 16,46 AND 81DAE IN SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE AND SUBJECTED TO DIFFERENT NITROGEN FERTILIZER CONCENTRATIONS IN THE SOIL (0,40,80,120 AND 160 KG HA^{-1})SIGNIFICANT AT P ≤0.05.

The carboxylation efficiency is an estimate between the CO_2 carboxylation and the internal CO_2 concentration in the mesophyll. Figure 3 shows the result of carboxylation efficiency.There was carboxylation efficiency decrease due to the increased nitrogen dose at 16 DAE. The efficiency response increased due to the increased dose in the N treatment at 46 DAE, and the increased N dose decreased the photosynthetic efficiency at 81 DAE.

Studies about the effects of the interaction between nitrogen availability and the isotopic fractionation of carbon 13 (13C) on the productivity of plants with C4 metabolism were conducted. The results found that the nitrogen deficiency dramatically reduced the photosynthetic capacity of sugarcane and corn, since the PEPC and rubisco activities were directly affected (RANJITH et al., 1995; MEIZER; ZHU, 1998).Kölln (2012) studied the photosynthetic metabolism of sugarcane and found 13C isotope decrease due to increased nitrogen availability. The author reported that such decrease may have resulted from the increased phosphoenolpyruvate carboxylase (PEPC) and rubisco activities in treatments with greater N availability.

The carboxylation efficiency in plants inoculated with Azospirilum brasilense showed different responses to the applied plant growth regulator level and influenced the nitrogen fertilization, as well as the assessment time (Figure 4).

According to the regression results, the dose of 466.6 mL ha^{-1} of plant growth regulator was estimated at 16 DAE in order to increase thephotosynthetic efficiency in plants that were not subjected to nitrogen fertilization. The A/Ciratio reached 8.00 µmol (CO_2) m^{-2} s^{-1}/ µmol (CO_2) mol^{-1} due to the higher net CO_2 assimilation and to the decreased internal CO_2 concentration.

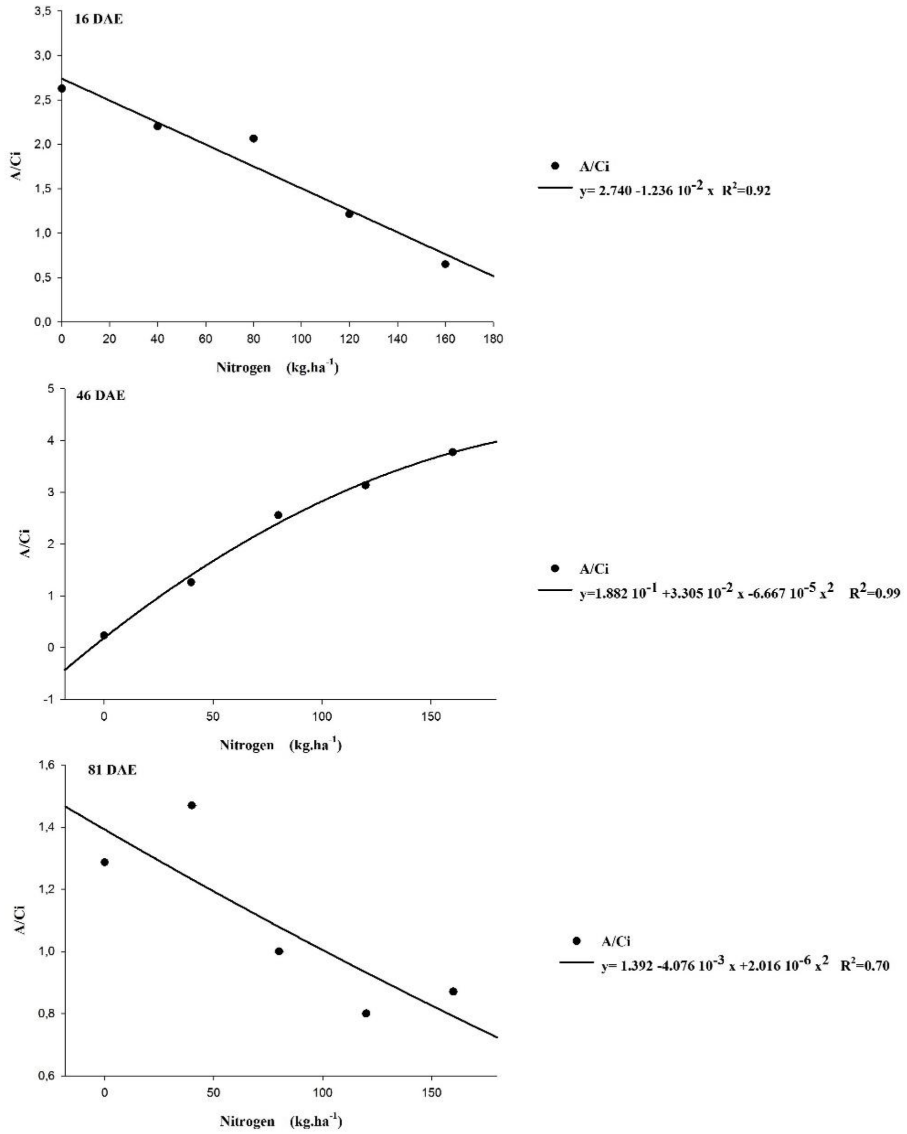

FIGURE 3. THE EFFECT OF NITROGEN CONCENTRATIONS (0, 40, 80, 120, AND 160 KG HA^{-1}) ON THE CARBOXYLATION EFFICIENCY (A/CI) AT 16,46 AND 81DAE IN SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE (*P ≤0.05).

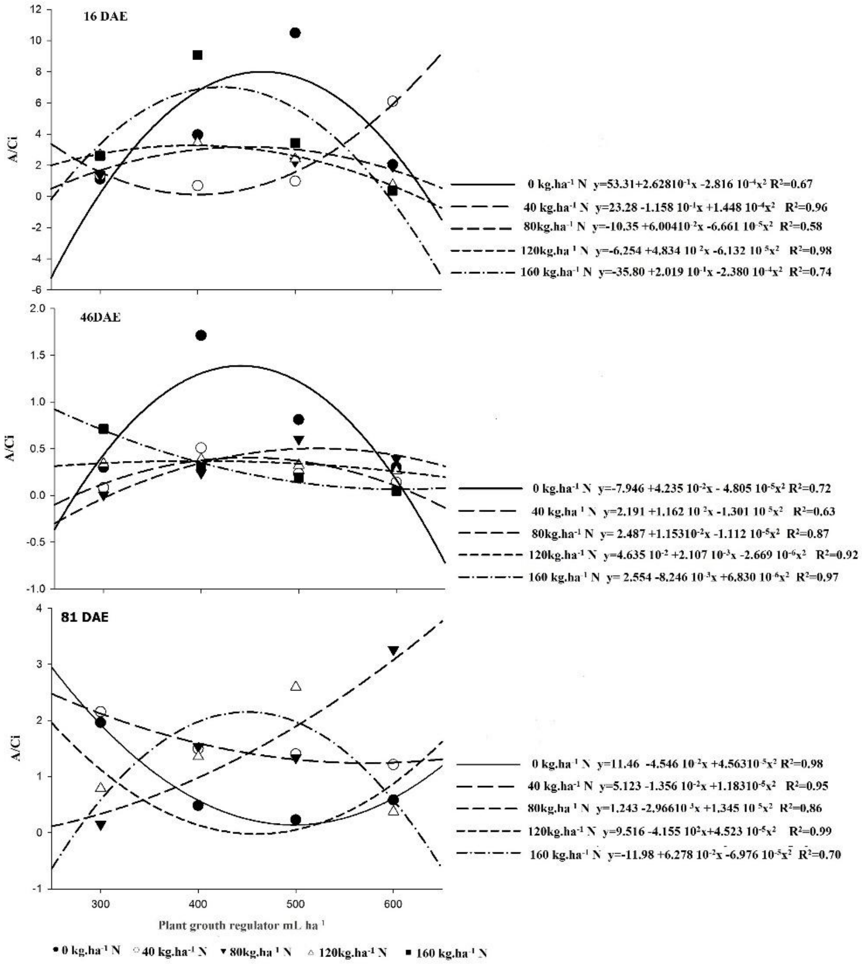

FIGURE 4. THE EFFECT OF PLANT GROWTH REGULATOR CONCENTRATIONS (300, 400, 500 and 600 mL ha^{-1}) ON THE CARBOXYLATION EFFICIENCY (A/CI) AT 16,46 AND 81DAE IN SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE AND SUBJECTED TO DIFFERENT NITROGEN FERTILIZATION CONCENTRATIONS (0,40,80,120 AND 160 KG HA^{-1}).SIGNIFICANT AT P ≤0.05.

There was increased carboxylation due to the inoculation with nitrogen-fixing bacteria and to the application of plant growth regulators from 16 to 46 DAE. The carboxylation efficiency was lower than that of the previous period; however, it was higher when it came to the treatments. In addition, the 440.68 mL dose of plant growth regulators provided 1.38 µmol(CO_2) m^{-2} s^{-1}/ µmol(CO_2) mol^{-1} efficiency in plants that were not fertilized with nitrogen.

According to Larcher (2006) and Barbosa Gonzaga (2012), the carboxylation efficiency is defined as the rate at which the fixed CO_2 is processed. This fixation rate mainly depends on the CO_2quantity and availability, as well as on the enzyme activity. It may also be influenced by the (RuBisCo) acceptor concentration, the temperature, the protoplasm hydration degree, the mineral substance supply (especially phosphate), and by the plant development and activity degree.

It may also be related to the higher carboxylation efficiency and to the longer *stomatal opening* permanence (LARCHER, 2006, Castro et al., 2005).

According to Criado et al. (2009), the exogenous application of cytokinins increases the rubisco enzyme activity and optimizes the carboxylation efficiency and the gibberellin by increasing the leaf area, and it may also influence the gas exchange. Thus, cytokinins and gibberellins have positive effect on plant growth and yield (ALBACETE; PÉREZALFOCEA, 2013; CRIADO et al., 2009).

According to Werner and Schmülling (2009), the cytokinin application increases the nitrate reductase enzyme activity. It indicates that the cytokinin also affects the nitrogen assimilation capacity of plants. According to Farquar and Sharkey (1982), high Ci values associated with low stomatal conductance would indicate decreased carboxylation efficiency.Saikia et al. (2007) found that wheat plants inoculated with *Azospirillum spp* showed higher photosynthesis rate and greater stomatal conductance, thus leading to increased seed yield, in comparison to non-inoculated plants.

According to Barros Neto (2008), the N levels did not influence the response to inoculation. Barraco et al. (2009) conducted studies on corn treated with *Azospirillum* and found 6% grain yield improvement in comparison to that of the non-inoculated control. Sala et al. (2008) inoculated wheat seeds and treated them with different nitrogen doses (0, 60 and 120 kg ha^{-1} of urea). They found responses to fertilization and to endophytic bacteria. The authors also reported that the increase in the nitrogen dose from 60 to 120 ha^{-1}led to linear efficiency ratio decrease in the use of this nutrient, which affected the crop growth components, as well as the photosynthetic production.

Table 1 shows the results of the mean squares of the technological parameters in the production of sugars - POL%, RS% and TRS (kg t^{-1}). It is worth highlighting that the N fertilization showed statistically significant difference in the POL% parameter among the treatments. However, the same result was not found after the application of the plant growth regulator treatments, thus corroborating the study by Silva et al. (2010), who assessed the effect of the application of Stimulate® associated with liquid fertilizers on the regrowth and productivity of the ratoon from five different sugarcane genotypes and found no significant effects of the plant growth regulator on the POL% and TRS analyses.

Table 1 also shows significant variation of the reducing sugar parameter (RS) in the interaction between the treatments with different doses of plant growth regulator and nitrogen.

TABLE 1

MEAN SQUARES OF THE VARIANCE ANALYSIS AND SIGNIFICANCE LEVELS; MEANS AND VARIATION COEFFICIENTS OF POL%,RS% AND TRS%, ACCORDING TO THE N CONCENTRATION AND PLANT GROWTH REGULATOR CONCENTRATION TREATMENTS APPLIED TO SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE.CAMPO GRANDE COUNTY,MS,2014/15.

Variation causes	POL%	RS%	TRS kg t^{-1}
N Doses	3.015*	0.021_{ns}	259.43*
PR Doses	0.379_{ns}	0.031_{ns}	21.95_{ns}
N x PR	0.076_{ns}	0.033*	3.88_{ns}
Residue	0.402	0.018	30.62
Overall Mean	10.44	0.60	94.0
C.V (%)	6.0	22.5	6.0

N Doses:Nitrogen Doses; PR Doses: Plant Growth Regulator Doses; N x PR: interaction between Nitrogen and Plant Growth Regulator doses; C.V: Coefficient of Variation. ^{ns}: non- significant; * Significant at $p \geq 0.05$.

According to Rocha et al. (2014), the largest accumulation of soluble sugars in the stem of cultivated plants due to their greater capacity to absorb N in its ammonia form. It is an advantage, in physiological terms, since the levels of soluble sugars in the plants indicate energy readily available for cell metabolism.

Ferreira et al. (2013) studied the effects applying the plant growth regulator Stimulate®(through plantation furrows) to eight sugarcane cultivars on the technological variables POL%, RS and TRS. They found lower percentage of reducing sugars in plants that were subjected to the treatment with plant growth regulator, fact that did not influence the other variables. The authors confirmed that the plant growth regulator stimulated the increased stem diameter and that the plants used sugars such as glucose and fructose to growth, which reduced the levels of these sugars.

The total recoverable sugar was not affected by the treatment with different plant growth regulator doses; however, the best response of the 96.27 kg t^{-1} TRS to the 86.73 kg ha^{-1} nitrogen was estimated in the treatment with different N doses (Figure 5).

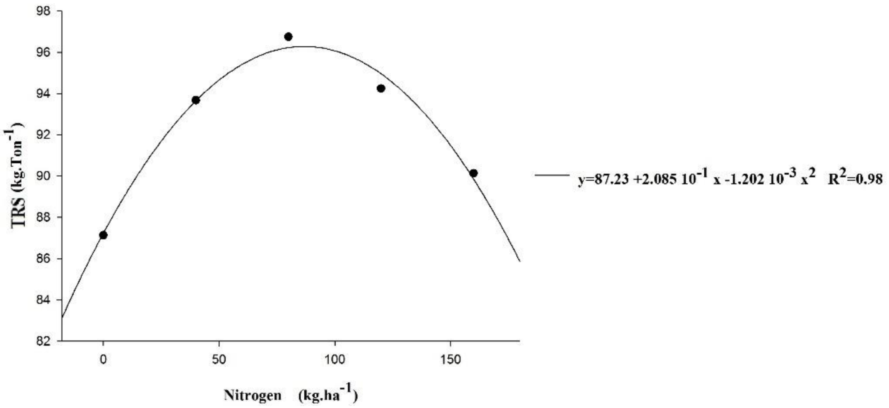

FIGURE5.TRS IN SWEET SORGHUM PLANTS INOCULATED WITH AZOSPIRILLUM BRASILENSE, ACCORDING TO THE NITROGEN DOSES.SIGNIFICANT AT P $\leq$0.05.

The TRS results, which were obtained according to the treatments, may be related to the fact that the photosynthetic activity in the leaf produces sugars and sugar alcohols, which tend to accumulate; such carbohydrates are osmotically active and diffuse into the vascular system in order to be transported to the phloem, through both active and passive mechanisms.The phloem loading mechanism may increase or restrict the carbon fixation and flux in response to the environmental conditions, and it may limit the strength of the sinkorgans, as well as the production of carbohydrates in the sources (SLEWINSKI; BRAUN, 2010).

According to Dalri and Cruz (2008) and Franco (2008), the N dose increase did not change the TRS in the sugarcane stems. Kölln (2012) found negative correlation between the increased N dose and the sugar content in the sugarcane stem. According to Sing and Mohan (1994), the sugar content decrease may be related to the fact that high nitrogen levels may increase the invertase enzyme activity; this enzyme degrades the reducing sugars converted into sugars (glucose and fructose). The N supply increases the C4 plant capacity to produce carbohydrates (biomass) and stimulates the plant growth, thereby diluting the concentration of sugars in the stem, which leads to the consequent industrial quality decrease (KÖLLN, 2012).

Since the non-reducing sugars are less reactive than their equivalents, they are transported over long distance in the phloem. The ketone or aldehyde group in the non-reducing sugars is reduced to alcohol or combined with a similar group in another sugar (DINANT; LEMOINE, 2010). Sucrose is the sugar predominantly transported in the phloem; however, mobile carbohydrates linked to a varying number of molecules such as raffinose, stachyose and verbascose, as well as sugar alcohols such as mannitol and sorbitol, are also transported in it (LALONDE et al.,2003; VAN BEL; HESS, 2008).

IV. CONCLUSION

The herein obtained results allow concluding that the plant growth regulator did not influence the production of sugars by the plant; however, it proved to be a good candidate to reduce the nitrogen fertilizationcosts in the culture, since it improved the photosynthetic efficiency in the sweet sorghum plants in which the metabolizedN derived from both the inoculation with *Azospirillum brasilense*and the nitrogen fertilization.

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