Australian Journal of Crop Science

AJCS

AJCS 14(12):1870-1875 (2020) ISSN:1835-2707
doi: 10.21475/ajcs.20.14.12.2590

Impact of N-fixing bacterium *Nitrospirillum amazonense* on quality and quantitative parameters of sugarcane in field condition

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Abstract

Sugarcane is the world's largest crop by production quantity. In Brazil, the sugarcane cultivation requires 30-70% less nitrogen than in other countries, due to the biological nitrogen fixation. *Nitrospirillum amazonense* is an N-fixing bacterium that has proven to increase plant growth and yields of sugarcane in greenhouse experiments. However, studies on field conditions are very scarce. For these reasons, this study aimed to assess the impact of different doses of a pre-commercial product, Aprinza®, containing *N. amazonense* on quality and quantitative parameters of the cultivar RB867515 in field conditions. The plant height, number of internodes, stem yield and sugar yield were measured. The leaf nutrient content was analyzed 60, 90 and 180 days after planting and the plant nutrient content was analyzed after harvest. The inoculation of *N. amazonense* did not affect the leaf and the stem nutrient content positively. The stem yield was increased 27.5 tons ha^{-1} (20%) and the total recoverable sugar yield increased 4.6 tons ha^{-1} (25%), compared to the control, by using 1 liter of Aprinza® per hectare. Therefore, *N. amazonense* can increase sugarcane stem and sugar yields in sandy soils with low nitrogen application, reducing the environmental impacts of the sugarcane cultivation system.

Keywords: *Nitrospirillum amazonense*; sugarcane; biological nitrogen fixation.

Introduction

Brazil produces about 30 billion liters of ethanol (26% of the total world production) (RFA, 2019) and 34.2 million metric tons of sugar (USDA, 2018) from sugarcane. It also provides biomass to supply 10.8% of the Brazilian electricity demand (MME, 2019). Sugarcane (*Saccharum spp.*) is a member of the Poaceae family and is the world's largest crop by production quantity, 1.8 billion tons in 2018 (FAO, 2019), providing around 70% of the world's sugar demand (Hofer, 2015). In 2018, Brazil produced 748 million tons of sugarcane in 10 million hectares and is the world's largest producer (FAO, 2019). Substantial amounts of nitrogen are required for commercial sugarcane cultivation due to large biomass production (Thorburn et al., 2005). In the USA, India, Colombia and Australia, 150 to 200 kg of nitrogen is applied per hectare per year. In comparison, Brazilian sugarcane fields require fertilization of 60 to 70 kg of nitrogen per hectare year (Urquiaga et al., 2012). Studies show that the relationships between the selected sugarcane genotype and bacterial strains can contribute to the biological nitrogen fixation, which provides 30 to 70% of the total nitrogen required for the sugarcane production cycle in the Brazilian production system (Urquiaga et al., 1992; Oliveira et al., 2002). A plethora of bacteria and fungi originated from the native soil are found inhabiting the exophytic and endophytic compartment of sugarcane roots, shoots and leaves, colonizing the plant organs in distinct patterns (Souza et al., 2016). Among these bacterial communities, endophytic diazotrophs bacteria are known for their ability to fix nitrogen (Boddey et al., 2003). A consortium of diazotrophic bacteria composed of five strains (*Glucanoacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Paraburkholderia tropica* and *Nitrospirillum amazonense*) increased the stem and sugar yield of the variety RB867515 in two different environments (Schultz et al., 2017). The same diazotrophic bacteria consortium increased sugarcane stem yield about 16% when compared to a negative control without fertilization and inoculation, not differing from a treatment with urea applied by fertigation in the Brazilian semi-arid region (Simões et al., 2019). In an hydroponic cultivation for 59 days, this bacteria consortium increased the biomass accumulation of the cultivar low fertility-adapted variety RB867515 (Santos et al., 2019a). The *Nitrospirillum amazonense*, previously known as *Azospirillum amazonense*, is a diazotrophic nitrogen-fixing bacterium (Lin et al., 2014) that was first isolated from Amazon region and in the state of Rio de Janeiro from cultivated grasses (Magalhaes et al., 1983). Studies with other members of the Poaceae family suggested positive effects of the inoculation of *N. amazonense*. Rodrigues et al. (2008) found that *N. amazonense* has a plant growth effect and can increase grain dry matter accumulation from 7 to 11.6% in rice, contributing to up to 27% of the nitrogen accumulation in plants. In experiments with wheat, *N. amazonense* produced responses of dry matter and N content (Boddey et al., 1986).

The inoculation of diazotrophic bacteria consortium can increase sugarcane yield without nitrogen fertilization in field conditions. Sole inoculation with *N. amazonense* showed to have the potential to increase plant growth and nitrogen concentration of the sugarcane seedlings shoot in greenhouse experiments. So far, no study has been reported on impacts of inoculation with *N. amazonense* alone on the qualitative characteristics and quantitative parameters of sugarcane in field conditions. Therefore, the aim of this study was to analyze the impact of applying different doses of *N. Amazonense* on stem yield and sugar yield, sugarcane growth and nutrients content of the leaves.

Results

Height of the last expanded leaf insertion and number of internodes

The height of the insertion of the last expanded leaf was measured at 90 days after planting and just before harvesting. At 90 days after application (DAP), treatments T2 and T5 were significantly higher, with 17.256 and 17.600 cm of insertion height, respectively. The control without microorganisms and fertilizer application (C1) was the one with the shortest height of the last expanded leaf insertion (14.080 cm). At this period, the mean of the treatments of *N. amazonense* was 16.194 cm. Before harvesting, T3 last expanded leaf insertion was significantly higher, 273.440 cm, while the controls C1 and C2 were significantly shorter, 251.740 and 252.196 cm, respectively. The insertion average of treatments of *N. amazonense* was 259.175 cm. The treatment T6, with commercial product Nemix® had no significant effect when compared to *N. amazonense* treatments and to both controls.

The number of internodes was quantified before harvesting and only the T3 had a significant effect, when compared to both controls (Table 2).

Stem yield and sugar yield

Treatments T3 (160.73 tons ha^{-1}), T1 (154.88 tons $ha^{-}1$) and T2 (149.91 tons ha^{-1}) stem yields were significantly higher than C1 (133.23 tons ha^{-1}). T1 stem yield was also significantly higher than C2 (135.74 tons ha^{-1}). Treatments T3 (22.704 tons ha^{-1}) and T1 (22.112 tons $ha^{-}1$) sugar yields were significantly higher than C1 (18.125 tons ha^{-1}). Treatment T3 was also significantly higher than C2 (19.237 tons ha^{-1}). The treatment with commercial product Nemix ® (T6) had no significant difference, when compared to both controls and the treatments of *N. amazonense* (Table 3).

Nutrient content in the leaf and stem

The macronutrient concentration of the first leaf was quantified at 60, 90 and 180 days after planting (DAP) and the macronutrient concentration of the stem was quantified 90 days after planting.

At 60 days after planting, there was a significant difference among the treatments for the concentrations of nitrogen, calcium, magnesium and sulfur in the first leaf. T1 nitrogen concentration in the first leaf (2.002%) was significantly lower than the concentrations of C2 (2.334%), T2 (2.454%) and T3 (2.328%). Treatment C2 calcium concentration in the first leaf (0.825%) was significantly higher than T3 (0.634%). Treatment C2 caused significantly higher magnesium concentration in the first leaf than T1 (0.324%), T2 (0.306%) and T6 (0.322%), and T5 sulfur concentration in the first leaf was significantly higher than T1 (0.120%) and T6 (0.116%). At 90 days after planting (DAP), there was a significant difference among treatments for the concentration of potassium in the first leaf. Treatments T1 (2.284%) and T2 (2.274%) were significantly higher than the T6 (1.906%). There were no significant effects of the treatments in the macronutrient concentration in the first leaf 180 days after planting (Table 4).

The proposed treatments with *N. amazonense* had no significant effects on stem's nitrogen, phosphorus, potassium, calcium and sulfur content, measured after harvesting. In control (C1) the magnesium concentration in the stem (0.254%) was significantly higher than the T2 (0.206%), T3 (0.214%), T4 (0.200%) and T6 (0.204%) (Table 5).

Discussion

Santos et al. (2017) studied effects of inoculation with different bacteria on cultivar RB966928 at several stages of cultivation of sprouting and tubes. They showed that *N. amazonense* was less effective in germination rate and biomass accumulation in the aerial part and in the roots, when compared to *Herbaspirillum rubrisubalbicans*, *Herbaspirillum seropedicae* and *Paraburkholderia tropica*, and the mixture of these bacteria. The inoculation of the bacteria consortium also had positive effects on sprouting and morphological traits in the early stages of the sugarcane development (Ferreira et al., 2020). Figueiredo et al. (2017) tested the effects of this consortium as plant growth promoting bacteria (PGPB) on six sugarcane genotypes in greenhouse experiments for 45 days. Two clones showed potential in PGPB studies and the RB867515 was non-responsive to the inoculation. However, these studies were carried out only in the early stages of the development of the plant. The *N. amazonense* significantly increased the height of the first leaf of the plant, 90 days after planting when applied in the doses of 0.5 L (T2) and 2.0 L ha^{-1} (T5).

Oliveira et al. (2002) studied seven different combinations of inoculum, using five endophytic diazotrophic species: *Glucanoacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Paraburkholderia tropica* and *Nitrospirillum amazonense*, originally isolated from sugarcane plants. They suggested that the application of the mixture of these five species was the best strategy to improve sugarcane crops dependent on the biological nitrogen fixation,

Table 1. Description of the treatments used in this experiment in relation to fertilization, type of microorganism applied and application dosage of the microorganisms.

Treatments	Fertilization*	Microorganisms applied	Dosage
C1	-	-	-
C2	Yes	-	-
T1	Yes	*N. amazonense*	0.25 L ha^{-1}
T2	Yes	*N. amazonense*	0.5 L ha^{-1}
T3	Yes	*N. amazonense*	1.0 L ha^{-1}
T4	Yes	*N. amazonense*	1.5 L ha^{-1}
T5	Yes	*N. amazonense*	2.0 L ha^{-1}
T6	Yes	*Bacillus subtilis + B. lichniformis*	1.0 kg ha^{-1}**

*It was applied 40, 40 and 60 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. **Standard commercial treatment, following the recommended application dose (Nemix ®).

Table 2. Impact of *N. amazonense* (EXP BR11145 U-AA) on the sugarcane last expanded leaf insertion height at 90 days after planting and harvest, and on the number of internodes before harvesting.

Parameter		Last expanded leaf insertion height (cm)				Number of internodes	
Time		90 DAP		Harvest		Harvest	
Treatments	C1	14.08	b	251.74	b	18.82	b
	C2	16.146	ab	252.196	b	18.446	b
	T1	15.854	ab	258.76	ab	19.04	ab
	T2	17.256	a	261.14	ab	19,04	ab
	T3	16.68	ab	273.44	a	20.134	a
	T4	16.174	ab	259.02	ab	18.8	ab
	T5	17.6	a	255.8	ab	19.418	ab
	T6	15.758	ab	261.3	ab	18.84	ab

Values in each column with different letters are significantly different at 0.1 significance level at Tukey test. C1 = control with no fertilization, C2 = control with fertilization, T1 = 0.25 L per hectare, T2 = 0.5 L per hectare, T3 = 1.0 L per hectare, T4 = 1.5 L per hectare, T5 = 2.0 L per hectare, and T6 = 1 kg per hectare of Nemix ®

Table 3. Impact of *N. amazonense* (EXP BR11145 U-AA) on the sugarcane yield and sugar yield.

Parameter		Stem yield (tons ha^{-1})		Total recoverable sugars yield (t ha^{-1})	
Treatments	C1	133.23	c	18.125	c
	C2	135.74	bc	19.237	bc
	T1	154.88	ab	22.112	ab
	T2	149.91	ab	20.778	abc
	T3	160.73	a	22.704	a
	T4	145.2	abc	20.902	abc
	T5	147.29	abc	20.947	abc
	T6	146.98	abc	21.336	abc

Values in each column with different letters are significantly different at 0.1 significance level at Tukey test. C1 = control with no fertilization, C2 = control with fertilization, T1 = 0.25 L per hectare, T2 = 0.5 L per hectare, T3 = 1.0 L per hectare, T4 = 1.5 L per hectare, T5 = 2.0 L per hectare, and T6 = 1 kg per hectare of Nemix ®

Table 4. Impact of *N. amazonense* (EXP BR11145 U-AA) in the first leaf concentration of nitrogen, phosphorus, potassium, calcium, magnesium and sulfur at 60, 90 and 180 days after planting.

Nutrient	Time	Treatments							
		C1	C2	T1	T2	T3	T4	T5	T6
N (%)	60DAP	2.100bc	2.334ab	2.002c	2.454ab	2.328ab	2.424abc	2.122bc	2.228abc
	90DAP^{ns}	2.296	2.516	2.564	2.420	2.274	2.520	2.374	2.480
	180DAP^{ns}	1.352	1.368	1.360	1.324	1.406	1.428	1.426	1.372
P (%)	60DAP^{ns}	0.380	0.382	0.444	0.446	0.380	0.436	0.382	0.366
	90DAP^{ns}	0.256	0.290	0.286	0.270	0.244	0.280	0.264	0.280
	180DAP^{ns}	0.236	0.240	0.252	0.248	0.258	0.244	0.246	0.234
K (%)	60DAP^{ns}	2.582	2.334	2.380	2.526	2.124	2.356	2.200	2.190
	90DAP	1.974ab	2.094ab	2.284a	2.274a	2.124ab	2.024ab	2.010ab	1.906b
	180DAP^{ns}	1.876	2.854	2.066	2.202	1.940	1.978	1.750	2.00
Ca (%)	60DAP	0.774ab	0.825a	0.686ab	0.710ab	0.634b	0,690ab	0.710ab	0.676ab
	90DAP^{ns}	0.496	0.556	0.520	0.444	0.490	0.570	0.496	0.576
	180DAP^{ns}	0.374	0.418	0.552	0.422	0.488	0.412	0.450	0.458
Mg (%)	60DAP	0.398ab	0.442a	0.324b	0.306b	0.370ab	0.360ab	0.374ab	0.322b
	90DAP^{ns}	0.224	0.234	0.246	0.206	0.216	0.244	0.234	0.236
	180DAP^{ns}	0.160	0.176	0.198	0.168	0.200	0.180	0.196	0.178
S (%)	60DAP	0.124ab	0.134ab	0.120b	0.136ab	0.134ab	0.144ab	0.160a	0.116b
	90DAP^{ns}	0.204	0.174	0.184	0.204	0.194	0.164	0.170	0.176
	180DAP^{ns}	0.142	0.150	0.158	0.166	0.160	0.146	0.174	0.160

Values in each column with different letters are significantly different at 0.1 significance level at Tukey test. n.s. = non significative effect. C1 = control with no fertilization, C2 = control with fertilization, T1 = 0.25 L per hectare, T2 = 0.5 L per hectare, T3 = 1.0 L per hectare, T4 = 1.5 L per hectare, T5 = 2.0 L per hectare, and T6 = 1 kg per hectare of Nemix ®

Table 5. Impact of *N. amazonense* (EXP BR11145 U-AA) in the stem concentration of nitrogen, phosphorus, potassium, calcium, magnesium and sulfur at 90 days after planting.

Treatments	N (%)^{ns}	P (%)^{ns}	K (%)^{ns}	Ca (%)^{ns}	Mg (%)		S (%)^{ns}
C1	2.576	0.322	2.244	0.606	0.254	a	0.190
C2	2.616	0.330	2.464	0.546	0.230	ab	0.206
T1	2.364	0.340	2.35	0.560	0.226	ab	0.180
T2	2.524	0.274	2.204	0.494	0.206	b	0.178
T3	2.524	0.284	2.37	0.504	0.214	b	0.174
T4	2.414	0.330	2.346	0.546	0.200	b	0.174
T5	2.416	0.306	2.386	0.584	0.220	ab	0.176
T6	2.334	0.294	2.344	0.490	0.204	b	0.184

Values in each column with different letters are significantly different at 0.1 significance level at Tukey test. n.s = non significative effects on N, P, K, Ca and S. C1 = control with no fertilization, C2 = control with fertilization, T1 = 0.25 L per hectare, T2 = 0.5 L per hectare, T3 = 1.0 L per hectare, T4 = 1.5 L per hectare, T5 = 2.0 L per hectare, and T6 = Nemix

contributing to around 30% of the total nitrogen accumulated in the plants of the variety SP70-1143. Oliveira et al. (2003), tested a bacteria consortium on 2 two micro propagated sugarcane varieties (SP 701143 and SP 813250) in three different locations in the Brazilian Center-South: Jau and Piracicaba in Sao Paulo and Seropedica, in Rio de Janeiro. This mixture of bacteria decreased the stem yield of SP 813250 variety, while a small increase was observed on yield of SP 701143. Oliveira et al. (2006) compared the soil conditions with the same varieties and at the same locations. The inoculation of diazotrophic bacteria increased the stem yield of the variety SP 701143 without nitrogen fertilization for three consecutive crops, and it was equivalent to the annual nitrogen fertilization. The application of synthetic nitrogen fertilizer emits greenhouse gases. Reducing nitrogen fertilization can substantially alter the overall greenhouse gas balance of a system (Smith and Conen, 2006). Thus, the inoculation of diazotrophic bacteria can make the sugarcane production system more sustainable. This fact deserves even more attention since the Brazilian Federal Government launched a program called RenovaBio. In the RenovaBio, biofuel producers will receive one financial title equivalent to carbon credits called CBIO, which corresponds to one ton of CO_2 that is no longer emitted due to the biofuel production. Fuels distributors will have an obligation to buy CBIOs and it will also be available to any interested investor (ANP, 2019; Grassi and Pereira, 2019).

Lopes et al. (2019) assessed the interaction between 27 sugarcane families and the consortium of 5 diazotrophic bacteria recommended by Oliveira et al. (2002, 2003, 2006) in two crop cycles. The 27 families of sugarcane presented positive and negative response to the inoculation of these bacteria. The response varied depending on the studied family, inoculant, and the evaluated characteristics. The inoculation of this consortium increased the yield of variety SP80-3280 by 10 tons ha^{-1}, when no nitrogen was applied. However, there was no significant difference among inoculation and nitrogen treatments, since the urea-N was the main N source of the plant (Antunes et al., 2019). It was in congruence with this study, where the inoculation of *N. amazonense* had positive impacts on the productive parameters, but did not affect the nitrogen concentration in the plant and leaves after 90 DAP. The magnesium was the only nutrient that had a significant difference in the stem content after harvest, reduced in treatments T2, T3, T4 and T6, compared to controls. Pedula et al. (2016) also found positive effects of these diazotrophic bacteria on the productive parameters of the variety RB92579 and the N, P and K accumulation, differently from the data obtained for *N. amazonense* in this study.

Shultz et al. (2017) analyzed the effects of applying the consortium of 5 diazotrophic bacteria on the stem yield and sugar quality of two varieties of sugarcane (RB867515 and RB72454) grown in two different environments in field conditions. The variety RB867515 (the same used in this study) was cultivated in Sapucaia-Rio de Janeiro, in the Brazilian Center-South. It showed the stem yield of 126.1 tons ha^{-1}, which was significantly higher, about 22.3 tons ha^{-1} more than the control, and 4.2 tons ha^{-1} less compared to fertilized with 120 kg of N ha^{-1}. The total recoverable sugar yield was increased from 14.34 tons ha^{-1} to 16.42 tons ha^{-1} when the bacteria consortium was inoculated and to 17.04 tons ha^{-1} in the treatment with N application. In Coruripe, in the Brazilian Northeast, the stem yield was increased from 115.9 to 153.9 tons ha^{-1} with the inoculation of bacteria, and to 158.3 tons ha^{-1} when nitrogen was applied. The total recoverable sugar yield was increased from 18.37 to 24.23 tons ha^{-1} and to 23.86 tons ha^{-1} when nitrogen was applied. The results of Schultz et al. (2017) study for the variety RB867515 (in the Brazilian Center-South region) were similar to the results found in this study, increasing by about 20% of the stem yield in both studies when the bacteria consortium and *N. amazonense* were applied. However, the most interesting parameter of the sugarcane production system is the sugar yield. In their study, the bacteria consortium increased the total recoverable sugar yield by 15%, while in this study, the *N. amazonense* alone increased the total recoverable sugar yield by 25%. The total nitrogen content in the plant was not significantly affected by any treatment in both studies.

The *N. amazonense* was beneficial for increasing stem yield when compared to control treatments. A similar result was also observed for sugar yield per hectare, and the 1.0L significantly exceeded the C1 (20.2%) and C2 (15.3%). Although it was not performed in field conditions, Santos et al. (2019b) predicted the potential to increase the plant growth and yield of RB867515, when diazotrophic bacteria are applied. In their study, the cultivar RB867515 showed no effect of inoculation for the content of P and K in the plant, similar to the results found in this study.

Materials and methods

Plant materials

The cultivar RB867515 is the most cultivated in Brazil, representing more than 22% of the total sugarcane cultivated area (Barbosa et al., 2012), because of its high production rates, high levels of sucrose and medium levels of fibers (Simões Neto et al., 2005). The clonal sets of this cultivar were from the State University of Maringá Experimental Farm.

The experimental site, environmental and soil conditions

The trial was installed at the Experimental Farm of Iguatemi belonging to the State University of Maringá (23 ° 21'9.91 "S and 52 ° 4'29.70" W, 541 meters altitude), in Maringá - PR, between May 2016 and August 2017. The prevailing climate in the municipality of the experiment was is the mesothermal Cfa - wet type, according to Köppen classification, with infrequent hot summers and frosts, a tendency for rainfall to occur in the summer months, without a defined dry season. The soil of the experimental area presented pH in H_2O of 6.50; 3.82 $cmol_c$ of CEC; 1.00 $cmol_c\ dm^{-3}$ of $H^+ + Al^{3+}$; 1.80 $cmol_c\ dm^{-3}$ of Ca^{2+}; 0.90 $cmol_c\ dm^{-3}$ Mg^{2+}; 0.12 $cmol_c\ dm^{-3}$ of K^+; 17.00 mg dm^{-3} of P; 1.40% of organic matter; 84.30% sand; 2.70% silt and 13.00% clay, with textural grade sandy.

Experimental design and treatments

The experimental design was randomized blocks with eight treatments and five replications. The treatments consisted of five different doses of inoculant EXP BR11145 U-AA / Aprinza® (0.25, 0.5, 1.0, 1.5, and 2 liters of solution with concentration of 10^8 colony-forming units of *N. amazonense*) applied directly in the planting furrow on sugarcane stalks, as well as a control without inoculation and without fertilization (C1), a control without inoculation and with fertilization (C2) and an industry standard treatment (IST) (Nemix C®, *Bacillus subtilis + B. lichniformis*). In all treatments, except C1, we applied 40 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O. The treatments were detailed in Table 1.
The experimental units consisted of five 10 m long sugarcane rows, with a total area of 75 m^2. In the evaluations and harvesting, 1.0 m from each end of the plots and a borderline were disregarded, with a total usable area of 36 m^2. The treatments were applied on May 18, 2016, in the sugarcane planting furrow, being sprayed on top of the sugarcane stalks, and subsequently covered with the soil. For the treatment applications, a CO_2-based constant pressure sprayer equipped with a TP-110.03 fan tip under pressure of 2 kg-force cm^{-2} was used. These application conditions provided the equivalent of 150 L ha^{-1} of solution.

Sugarcane quantitative analysis

After application, the following response variables were evaluated: insertion height of the last expanded leaf at harvesting time, leaf area and shoot dry mass evaluations were performed (90 DAP), collecting these observations from fifteen plants per experimental unit.
At 180 DAP, the yield of sugarcane stems was estimated. This value was obtained by weighing ten stems and then multiplying the average weight of each stem by the number of tillers contained in one hectare. The number of internodes was counted at harvesting time (average of fifteen plants previously identified per plot). Sugarcane was harvested manually on 08/08/2017, cutting the stems present in the plot's useful area (38.4 m^2) and the total weight subsequently converted to production per hectare (tons ha^{-1}) and tons of sugars per hectare based on sugarcane technological analyzes made in the laboratory Cooperval Sugarcane Mill Industry, following the Manual Consecana (Consecana, 2012).

Qualitative analysis

The macronutrient contents (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) in leaf limb (without vein) of leaf +1 (20 plants per plot) were evaluated at 60, 90 and 180 DAP. For the evaluation of nutrient contents of the sugarcane leaf tissue, the collected leaves were sent for analysis at the Unithal Laboratório Agronômico, following the recommendations by Malavolta et al. (1997). At 90 DAP, the macronutrient contents of the sugarcane canopy (leaf + stem) (15 plants per plot) were also evaluated (Malavolta et al., 1997).

Statistics

The data were subjected to analysis of variance by the F test at 10% probability, using the software R. When they were significant, the means were compared by the Tukey test, also at 10% probability.

Conclusions

None of the treatments containing the EXP BR11145 U-AA / Aprinza® inoculant had negative effects on the growth, development, yield of the cultivar RB 867515 in sandy-textured soil and had positive effects on several of the evaluated response variables. Significant differences were observed in plant height, the number of internodes, nutrient content in the leaf, stem yield and sugar yield per hectare.
Treatment T3, with the application of 1.0L of Aprinza® per hectare to sugarcane tillage and furrow cultivated in sandy soil, significantly increased the yield of stalks compared to non-inoculated and non-fertilized control treatments and did not differ from the Nemix commercial standard. Therefore, the EXP BR11145 U-AA / Aprinza® containing 10^8 colony-forming units can be applied in a dosage of 1.0L of commercial product per hectare, in a 150 liters solution, to increase stem yield and sugar yield of sugarcane variety RB 86-7515 in sandy soils.

References

Antunes JEL, Freitas ADS, Oliveira LMS, Lyra MDCCP, Fonseca MAC, Santos CERS, Oliveira JP, Araújo ASF, Figueiredo MVB (2019) Sugarcane inoculated with endophytic diazotrophic bacteria: Effects on yield, biological nitrogen fixation and industrial characteristics. An Acad Bras Cien. 91(4): 1-12.

ANP - Brazilian National Agency of Petroleum, Natural Gas and Biofuels (2019) Renovacalc. http://www.anp.gov.br/producao-de-biocombustiveis/renovabio/renovacalc. (Accessed November 10, 2019).

Barbosa MHP, Resende MDV, Dias LAS, Barbosa GVS, Oliveira RA, Peternelli LA, Daros E (2012) Genetic improvement of sugar cane for bioenergy: the brazilian experience in network research with RIDESA. Crop Breed Appl Biotechnol. S2: 87–98.

Boddey RM, Baldani VLD, Baldani JI, Döbereiner J (1986) Effect of inoculation of Azospirillum spp. on nitrogen accumulation by field-grown wheat. Plant Soil. 95(1): 109–121.

Boddey RM, Urquiaga S, Alves BJR, Reis V (2003) Endophytic nitrogen fixation in sugarcane: Present knowledge and future applications. Plant Soil. 252(1): 139–149.

CONSECANA - Sugarcane, Sugar and Ethanol Producers Council of the State of São Paulo (2012) Instruction Manual. http://consecana.sistemafaep.org.br/wp-content/uploads/2016/02/Manual-Consecana-2012.pdf (Accessed November 10, 2019).

FAO - Food and Agriculture Organization (2019) FAOSTAT. http://www.fao.org/faostat/en/#data/QC. (Accessed November 10, 2019).

Ferreira NS, Matos GF, Meneses CHSG, Reis VM, Rouws JRC, Schwab S, Baldani JI, Rouws LFM (2020) Interaction of phytohormone-producing rhizobia with sugarcane mini-setts and their effect on plant development. Plant Soil. 1–18.

Figueiredo GGO, Civiero JC, Romano T, Bespalhok Filho JC, Daros E (2017) Sugarcane clones inoculated with five species ofpromoting growth plant bacteria. Idesia (Arica) 35(2): 1–6.

Grassi MCB, Pereira GAG (2019) Energy-cane and RenovaBio: Brazilian vectors to boost the development of Biofuels. Ind Crop Prod. 129: 201-205.

Hofer R (2015) Sugar- and Starch-Based Biorefineries. Ind Biorefineries White Biotechnol. 4: 157–235.

Lin SY, Hameed A, Shen FT, Liu YC, Hsu YH, Shahina M, Lai WA, Young CC (2014) Description of Niveispirillum fermenti gen. nov., sp. nov., isolated from a fermentor in Taiwan, transfer of Azospirillum irakense (1989) as Niveispirillum irakense comb. nov., and reclassification of Azospirillum amazonense (1983) as Nitrospirillum amazonense gen. nov. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 105(6): 1149–1162.

Lopes VR, Bespalhok Filho JC, Figueiredo GGO, Oliveira RA, Daros E (2019) Interaction between sugarcane families and plant growth-promoting bacteria in two crop cycles. Semina: Ciências Agrárias. 40(2): 527–538.

Magalhaes FM., Baldani JI., Souto SM., Kuykendall JR., Dobereiner J (1983) New acid-tolerant Azospirillum species . An Acad Bras Cien. 55: 417–430.

Malavolta E, Vitti GC, Oliveira SA (1997) Evaluation of plant nutritional status: Principles and applications. 1st edn. Potafos, Piracicaba.

MME - Brazilian Ministry of Mines and Energy (2019) National Energy Balance based on 2018. http://www.epe.gov.br/sites-pt/publicacoes-dados-abertos/publicacoes/PublicacoesArquivos/publicacao-377/topico-470/Relatório Síntese BEN 2019 Ano Base 2018.pdf. (Accessed February 25, 2020).

Oliveira ALM, Urquiaga S, Döbereiner J, Baldani JI (2002) The effect of inoculating endophytic N2-fixing bacteria on micropropagated sugarcane plants. Plant Soil 242: 205–215.

Oliveira ALM, Canuto EL, Reis VM, Baldani JI (2003) Response of micropropagated sugarcane varieties to inoculation with endophytic diazotrophic bacteria. Brazilian J Microbiol. 34: 59–61.

Oliveira ALM, Canuto EL, Urquiaga S, Reis VM, Baldani JI (2006) Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. Plant Soil 284: 23–32.

Pedula RO, Schultz N, Monteiro R, Pereira W, Araujo PA, Urquiaga S, Reis VM (2016) Growth analysis of sugarcane inoculated with diazotrophic bacteria and nitrogen fertilization. African J Agri Res. 11(30): 2786–2795.

RFA - US Renewable Fuels Association (2019) Annual ethanol production. https://ethanolrfa.org/statistics/annual-ethanol-production/. (Accessed November 12, 2019)

Rodrigues EP, Rodrigues LS, Oliveira ALM, Baldani VLD, Teixeira KRDS, Urquiaga S, Reis VM (2008) Azospirillum amazonense inoculation: Effects on growth, yield and N 2 fixation of rice (Oryza sativa L.). Plant Soil. 302(1-2): 249–261.

Santos SG, Ribeiro FS, Fonseca CS, Pereira W, Santos LA, Reis VM (2017) Development and nitrate reductase activity of sugarcane inoculated with five diazotrophic strains. Arch Microbiol. 199(6): 863–873.

Santos SG, Chaves VA, Ribeiro FS, Alves GC, Reis VM (2019a) Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria. Appl Soil Ecol. 133: 12–23.

Santos SG, Ribeiro FS, Alves GC, Santos LA, Reis VM (2019b) Inoculation with five diazotrophs alters nitrogen metabolism during the initial growth of sugarcane varieties with contrasting responses to added nitrogen. Plant Soil. 1–20.

Schultz N, Pereira W, Silva PA, Baldani JI, Boddey RM, Alves BJR, Urquiaga S, Reis VM (2017) Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium. Plant Prod Sci. 20(4): 366–374.

Simões Neto DE, Melo L, Chaves A, Lima R (2005) Launch of new RB varieties of sugar cane (. Recife: Imprensa Universitária/UFRPE.

Simões WL, Oliveira AR de, Souza MA de, Reis VM, Ivan Fernandes-Júnior P (2019) Efficient inoculation of diazotrophic bacteria into sugarcane by the drip irrigation system. Pesqui Agropecuária Bras. 54: e01110

Souza RSC, Okura VK, Armanhi JSL, Jorrín B, Lozano N, Silva MJ, González-Guerrero M, Araújo LM, Verza NC, Bagheri HC, Imperial J, Arruda P (2016) Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. Nature Sci Rep. 6(1): 1–15.

Smith KA, Conen F (2006) Impacts of land management on fluxes of trace greenhouse gases. Soil Use Manag. 20(2): 255–263.

Thorburn PJ, Meier EA, Probert ME (2005) Modelling nitrogen dynamics in sugarcane systems: Recent advances and applications. F Crop Res. 92(2-3): 337–351.

Urquiaga S, Cruz KHS, Boddey RM (1992) Contribution of nitrogen fixation to sugar cane. Nitrogen-15 and nitrogen-balance estimates. Soil Sci Soc Am J. 56(1): 105–114.

Urquiaga S, Xavier RP, de Morais RF, Batista RB, Schultz N, Leite JM, Maia e Sá J, Barbosa KP, de Resende AS, Alves BJR, Boddey RM (2012) Evidence from field nitrogen balance and 15N natural abundance data for the contribution of biological N 2 fixation to Brazilian sugarcane varieties. Plant Soil. 356(1-2): 5–21.

USDA – United States Department of Agriculture (2018) Sugar: Wold Markets and Trades. http://www.fas.usda.gov/commodities/sugar (Accessed November 12, 2019).