DOI: https://doi.org/10.17921/1415-6938.2021v25n3p295-301

Azospirillum Brasilense Application in Pre-Sprouted Seedlings (PSS) of Sugarcane

Aplicação de *Azospirillum Brasilense* em Mudas Pré-Brotadas (MPB) de Cana-de-Açúcar

Andressa Santos da Costa*[a]; Fábio Steiner[a]; Sara da Silva Abes[a]; Daniel Henrique Santana Rodrigues[a]; Kátia Cristina da Silva[a]; Isabela Machado de Oliveira Lima[a]

[a]Universidade Estadual de Mato Grosso do Sul, Curso de Graduação em Agronomia. MS, Brasil.
*E-mail: andressasantos4@hotmail.com

Abstract

The use of sugarcane pre-sprouted seedlings (PSS) of high quality is a key factor for success of sugarcane production system in Brazil, and the use of plant growth-promoting bacteria (PGPB) can be decisive in obtaining high-quality seedlings. However, it is not known which rate of inoculant containing *Azospirillum brasilense* should be used. Thus, this study was conducted to evaluate the efficiency of *Azospirillum brasilense* inoculation and establish the optimal rate of inoculant application on the development of sugarcane PSS (*Saccharum officinarum*). Pre-sprouted seedlings from commercial hybrids IACSP95-5000, RB855035, RB855536, and RB867515, produced from individual buds (mini-sets) of 3.0 cm were transplanted at 12 days after sprouting into 290 mL tubes. Treatments were arranged in completely randomized design, in 4 × 5 factorial scheme: four genotypes (IACSP95-5000, RB867515, RB855035 and RB855536) and five rates of commercial inoculant AzoTotal®, containing the strains AbV5 and AbV6 [0 (control); 0.5; 1.0; 2.0 and 4.0 mL per tube], four replicates. The inoculant rates increased the plant height, shoot dry matter, root dry matter, total dry matter, height: diameter ratio, and Dickson quality index of PSS of the genotypes IACSP95-5000, RB855035 and RB867515, as well as an increase in number of leaves and stalk diameter of RB855035 and RB867515. The genotype RB855536 has no response to application of inoculant containing *A. brasilense* during the production phase of pre-sprouted seedlings. The optimal application rate of inoculant containing *A. brasilense* for production of high-quality pre-sprouted sugarcane seedlings varies between 2 and 3 mL of inoculant per seedling.

Keywords: *Saccharum Officinarum*. Diazotrophic Bacteria. Nitrogen. Inoculation.

Resumo

O uso de mudas pré-brotadas (MPB) de cana-de-açúcar (Saccharum officinarum) de qualidade é fator chave para sucesso da produção de cana-de-açúcar no Brasil e o uso de bactérias promotoras de crescimento de plantas pode ser determinante na obtenção de mudas de qualidade. Entretanto, não se tem conhecimento da dose ideal de inoculante contendo Azospirillum brasilense. Este estudo foi conduzido com o objetivo de avaliar a eficiência da inoculação de Azospirillum brasilense e estabelecer a dose ideal no desenvolvimento de MPB de cana-de-açúcar. MPB de quatro variedades de cana-de-açúcar, oriundas de minirrebolos de 3,0 cm foram transplantadas aos 12 dias após a brotação para tubetes de 290 mL. Os tratamentos foram dispostos em delineamento casualizado, esquema fatorial 4 × 5: quatro variedades (IACSP95-5000, RB867515, RB855035 e RB855536) e cinco doses do inoculante comercial AzoTotal®, contendo as estirpes AbV_5 e AbV_6 [0 (controle); 0,5; 1,0; 2,0 e 4,0 mL por tubetes], com quatro repetições. As doses de inoculantes resultaram no aumento da altura das plantas, matéria seca da parte aérea, matéria seca das raízes, matéria seca total, relação altura: diâmetro e no índice de qualidade de Dickson das MPB das variedades IACSP95-5000, RB855035 e RB867515, além de resultar no aumento do número de folhas e diâmetro do colmo das variedades RB855035 e RB867515. A variedade RB855536 não responde à aplicação de inoculante durante a fase de produção de MPB. A dose ótima do inoculante para na produção de MPB de qualidade pode variar entre 2 e 3 mL de inoculante por muda.

Palavras-chave: *Saccharum Officinarum. Bactérias Diazotróficas. Nitrogênio. Inoculação.*

1 Introduction

Sugarcane (*Saccharum officinarum*) is an important industrial crop and is conventionally propagated through vegetative multiplication with the use of stalk segments called sets containing two to three buds. However, this conventional propagation system has used a high amount of vegetative material, resulting in less stalk availability for the sugar and ethanol production industry. In general, the amount of vegetative material used in the manual and mechanized planting system in Brazil has been in the order of 12 and 20 Mg ha^{-1} of stalks (SANTOS; BORÉM 2013).

In this context, new alternatives to reduce the amount of vegetative material required for planting commercial areas and the renovation of sugarcane plantations have been investigated by the Brazilian sugarcane sector, aiming at increasing efficiency and the farmers' economic gains.

The system of sugarcane pre-sprouted seedlings (PSS) is a new multiplication technology that contributes to the rapid seedlings production and is associated with a high standard of vegetal health, vigor and uniformity of planting, as well as a drastic reduction in the use of vegetative material and seedlings per area (LANDELL *et al*., 2012; AQUINO *et al*., 2018; ALMEIDA-NETO *et al*., 2020).

However, the quality of the pre-sprouted seedling used is a key factor for the success of the Brazilian sugarcane production system. During the seedling production phase, the type and size of the container, the physicochemical characteristics of the substrates and the use of products that promote the rooting of the seedlings are important factors that can affect the quality of the sugarcane seedling (OLIVEIRA *et al*., 2018; BRAGA *et al*., 2019; SANTOS *et al*., 2019; TEIXEIRA *et al*., 2020; FRANCO *et al*., 2020).

The use of plant growth-promoting bacteria (PGPB) has the potential to improve plant development and the quality of sugarcane seedlings (PEREIRA *et al*., 2013; GÍRIO *et al*., 2015; OLIVEIRA *et al*., 2018). PGPB may promote plant growth by regulating plant hormones, improve nutrition acquisition and symbiotic nitrogen fixation, and siderophore production, and enhance the antioxidant system (INAGAKI *et al*. 2014; SOUZA *et al*., 2015; SHIN *et al*., 2016; FUKAMI *et al*., 2018; LOPES *et al*., 2019). Also, some PGPB may infer more specific plant growth-promoting traits, such as drought tolerance (AGAMI *et al*., 2016).

Bacteria of the *Azospirillum* genus are, certainly, the most employed and studied PGPB in Brazil and worldwide (MARKS *et al*. 2013; HUNGRIA *et al*. 2015; BULEGON *et al*. 2017). *Azospirillum brasilense* is a gram-negative endophytic bacterium, capable of fixing N and colonizing all parts of the plant, especially the roots of a wide variety of grass species (BASHAN; HOLGUIN, 1997).

In addition to atmospheric N_2 fixation capacity, this bacterium contributes to greater plant development through numerous mechanisms, such as the synthesis of growth-promoting substances, especially auxin, gibberellin and cytokinin, increased activity of the enzyme nitrate reductase, induction of plant resistance to abiotic and biotic stresses, synthesis of siderophores and solubilization of phosphates (FUKAMI *et al*., 2018).

Studies have proven the effectiveness of *Azospirillum brasilense* inoculation in sugarcane crop, resulting in greater plant development and higher stalk productivity (MOUTIA *et al*., 2010; SERNA-COCK *et al*., 2011; MOURA *et al*., 2019; FRANCO *et al*., 2020); however, the effect of inoculation of *A. brasilense* on the pre-sprouted seedlings formation is unknown. Besides, the response to the inoculation of *A. brasilense* depends on the sugarcane genotype and the plants' growth conditions (MOUTIA *et al*., 2010; SCHULTZ *et al*., 2012; URQUIAGA *et al*., 2012; PEREIRA *et al*., 2013).

Gírio *et al*. (2015) reported that the *A. brasilense* inoculation improved the sprouting rate and dry matter accumulation of the seedlings in some of the sugarcane genotypes. In turn, Gonçalves *et al*. (2020) reported that the use of *A. brasilense* in pre-sprouted sugarcane seedlings has a beneficial effect on the plants' initial development only when associated with N fertilizer application. Besides, these authors reported that the use of 4 mL per seedling of inoculant containing *A. brasilense* caused phytotoxic effects on the development of sugarcane plants up to 60 days after transplanting. These contrary results show that other studies should be carried out, not only to investigate the effects of *A. brasilense* in different sugarcane genotypes but also to establish what the optimal rate to be applied on the pre-sprouted seedlings is.

This study was conducted to investigate the efficiency of *Azospirillum brasilense* inoculation and to establish the optimal rate of inoculant application on the development and quality of pre-sprouted seedlings (PSS) of sugarcane (*Saccharum officinarum*) genotypes.

2 Material and Methods

The experiment was carried out under greenhouse conditions at State University of Mato Grosso do Sul, in Cassilândia, Mato Grosso do Sul, Brazil (19º05'30" S; 51º48'50" W and altitude of 540 m), from October to December 2018. Temperature and relative humidity data were gathered daily using a datalogger (ITLOG-80, Instrutemp Measuring Instruments Ltd., São Paulo, SP, BRA) installed inside the greenhouse. The photosynthetic photon flux density (PPFD) was measured daily at midday (± 12:00 h) with a photosynthetically active radiation meter (APG-MQ-100, Apogee Instruments Ltda., Piracicaba, SP, BRA). The environmental conditions during the experiment were: mean air temperature of 28.6 ºC during the day and 23.4 ºC during the night, average air relative humidity of 72% (±5%) and midday photosynthetic photon flux density of 1,178 $\mu mol\ m^{-2}\ s^{-1}$ (±217 $\mu mol\ m^{-2}\ s^{-1}$).

Individual buds (mini-setts) of 3.0 cm in size, as recommended by Franco *et al.* (2020) were extracted from the middle third of the stalks at 11 months of age from commercial hybrids IACSP95-5000, RB855035, RB855536, and RB867515. The sprouting process of one node is called Pre-Sprouted Seedlings (PSS) and is commonly used in the sugarcane cropping system in Brazil. These genotypes were used because they are the main commercial sugarcane hybrids grown in the Brazilian Cerrado region.

A completely randomized experimental design arranged in a 4 × 5 factorial scheme with four replications was used. Treatments consisted of four sugarcane genotypes (IACSP95-5000, RB855035, RB855536 and RB867515) and five application rates of the inoculant containing *A. brasilense* [0 (control); 0.5; 1.0; 2.0 and 4.0 mL per tube]. Each experimental unit consisted of five tubes containing a sugarcane seedling, with a total of 500 seedlings.

The seedling inoculation with *Azospirillum brasilense* was performed with the commercial liquid inoculant AzoTotal® (Total Biotechnology), which contains the strains AbV5 and AbV6 [minimum concentration of 2.0 × 10^8 colony-forming units (CFU) per mL]. The inoculant was applied on the roots, when the seedlings were transplanted to the tubes, using a pipette graduated in milliliters.

Pre-sprouted sugarcane seedlings in plastic boxes (42 × 28 × 6 mm) were transplanted 12 days after sprouting into 290 mL plastic tubes, filled with Carolina Soil® commercial substrate. The substrate had the following characteristics: pH in water 5.6; 0.85 g dm^{-3} of N; 0.18 g dm^{-3} of P, 0.25 g dm^{-3} of K; 1.24 g dm^{-3} of Ca; 0.72 g dm^{-3} of Mg; 0.30 g dm^{-3} of S; 0.70 dS m^{-1} of electrical conductivity (CE); 76% of total porosity and 55% of water retention capacity. After transplanting, all the sugarcane seedlings were grown in a protected environment and daily irrigated with a micro-sprinkler irrigation system, using an 8.0 mm irrigation depth.

At 60 days after transplanting, the sugarcane seedlings were removed from the tubes, and the roots washed under running water to remove the substrate. The plant height (PH), number of leaves (NL), stalk diameter (SD), and dry matter of the plant parts were measured. The seedlings were separated into leaves, stalks, and roots, oven-dried at 65 ºC for three days, and then weighed. The shoot dry matter (SDM) was obtained from the sum of the dry matter of the leaves and stalks. The total dry matter (TDM) was obtained from the sum of all the seedling parts (leaves, stalks, and roots). From these measurements the following parameters were calculated height: diameter ratio [HDR; plant height (cm)/stalk diameter (mm)], shoot: root dry matter ratio [SRR; shoot dry matter (g)/ root dry matter (g)], and Dickson quality index [DQI = TDM/ (HDR + SRR] (DICKSON *et al*., 1960).

The normality of data was previously tested by the Kolmogorov-Smirnov test at the 5% level and then the data were submitted to analysis of variance (ANOVA). The means of the sugarcane genotypes were compared by the Tukey test at the level of 5% probability. Regression analysis was used for the application rates of inoculant containing *A. brasilense* and significant equations (F-test, $p \leq 0.05$) with the highest coefficients of determination were adjusted. All the analyses were performed using Sisvar® software, version 5.6 for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

3 Results and Discussion

The results of the analysis of variance showed significant effects ($P < 0.05$) for the effects of sugarcane genotypes and application rates of *A. brasilense*, as well as for the interaction between these factors, for many of the measured traits (Table 1). The significant interaction between the effects of genotypes and *A. brasilense* rates indicates that sugarcane genotypes have a distinct response to the application rates of inoculant containing *A. brasilense*.

Table 1 - Summary of the analysis of variance for the measurements of morphological traits of sugarcane (*Saccharum officinarum*) genotypes affected by the application of inoculant containing *Azospirillum brasilense*

Genotype	**PH**	**NL**	**SD**	**SDM**	**RDM**	**TDM**	**HDR**	**SRR**	**DQI**
	(cm)		**(mm)**	**(g)**	**(g)**	**(g)**			
IACSP95-5000	52.0 c	7.85 a	5.65 b	3.15 a	1.22 c	4.36 b	9.25 c	2.79 a	0.37 b
RB855035	66.7 a	7.18 b	6.67 a	3.45 a	1.55 b	5.00 a	10.08 ab	2.33 a	0.41 ab
RB855536	60.7 b	7.29 b	6.15 ab	3.43 a	1.84 a	5.27 a	9.90 b	1.94 b	0.45 a
RB867515	58.1 b	6.92 b	5.62 b	3.25 a	1.31 c	4.55 b	10.48 a	2.54 a	0.36 b
Causes of variation	**Probability > F**								
Genotype (G)	<0.000	<0.000	<0.000	0.209	<0.000	0.004	<0.000	<0.000	0.002
Inoculant rate (I)	<0.000	0.605	<0.000	<0.000	0.006	<0.000	0.081	0.185	<0.000
Interaction G × I	0.001	<0.000	<0.000	0.047	0.031	0.042	<0.000	0.102	0.029
CV (%)	9.06	6.10	11.16	16.14	26.23	17.45	5.69	21.76	20.04

PH: plant height. NL: number of leaves. SD: stalk diameter. SDM: shoot dry matter. RDM: root dry matter. TDM: total dry matter. HDR: height: diameter ratio. SRR: shoot: root dry matter ratio. DQI: Dickson quality index. Mean followed by distinct letter, in the column, for the sugarcane genotypes show significant differences (Tukey test, $p \leq 0.05$). CV: coefficient of variation.
Source: Research Data.

The plant height (PH) was significantly higher in the RB855035 genotype, followed by the genotypes RB855536 and RB867515, and lower for the genotype IACSP95-5000 (Table 1). The number of leaves (NL) was significantly higher in the genotype IACSP95-5000. The stalk diameter (SD) was significantly higher in the genotype RB855035 and lower in the genotypes RB867515 and IACSP95-5000. The shoot dry matter (SDM) was similar for all the four sugarcane genotypes.

The root dry matter (RDM) was significantly higher in the genotype RB855536, followed by the genotype RB855035, and lower for the genotypes RB867515 and IACSP95-5000. Total dry matter (TDM) was significantly higher in the genotypes RB855035 and RB855536. The plant height: stalk diameter ratio (HDR) was significantly higher in the genotype RB867515, followed by the genotypes RB855035 and RB855536, and lower for the genotype IACSP95-5000.

The shoot: root dry matter ratio (SRR) was significantly higher in the genotypes IACSP95-5000, RB855035, and RB867515. The Dickson quality index (DQI) was significantly higher in the genotype RB855536 and lower for the genotypes IACSP95-5000 and RB855536 (Table 1). The differences in morphological traits between the sugarcane genotypes are due to the genetic differences of these genotypes.

The inoculant rates containing *A. brasilense* applied resulted in a linear increase in plant height of the genotype IACSP95-5000, while the highest plant height of the genotypes

RB855035 and RB 867515 was obtained with the application of 3.1 and 2.6 mL inoculant per seedling, respectively (Table 2). The number of leaves per plant increased linearly with the inoculant rates applied to the genotype RB867515, while the highest number of leaves of the genotype RB855035 was obtained with the application of 2.1 mL of inoculant per seedling. The highest stalk diameter for genotypes RB855035 and RB867515 were obtained with the application of 2.6 and 2.8 mL of seedlings per seedling, respectively. However, the genotypes IACSP95-5000 and RB855536 had no response to the application of inoculant containing *A. brasilense* for the number of leaves and stem diameter (Table 2).

Table 2 - Coefficients of the regression equations and determination coefficients (R^2) for the polynomial regression analyzes, optimal inoculant rate and maximum estimated value (Y_E) for the plant height, number of leaves and stalk diameter of the four sugarcane genotypes inoculated with *A. brasilense*

Genotype	Regression coefficients			R^2	*P*	Optimal inoculant rate (mL/seedling)	Maximum value (Y_E)
	Intercept (β_0)	β_1	β_2				
	Plant height (cm)						
IACSP95-5000	49.2	1.84		0.57	0.035	–	–
RB855035	56.9	12.18	–1.985	0.64	0.011	3.1	75.6
RB855536	60.7				0.158	–	–
RB867515	48.7	13.89	–2.696	0.96	0.001	2.6	66.6
	Number of leaves						
IACSP95-5000	7.84				0.073	–	–
RB855035	6.80	0.76	–0.178	0.76	0.006	2.1	7.59
RB855536	7.29				0.278	–	–
RB867515	6.48	0.29		0.69	0.001	–	–
	Stalk diameter (mm)						
IACSP95-5000	5.65				0.616	–	–
RB855035	5.36	1.92	–0.367	0.66	0.001	2.6	7.96
RB855536	6.15				0.289	–	–
RB867515	4.24	1.86	–0.332	0.97	0.001	2.8	6.84

P: Probability of the F test for the significance of the regression model.
Source: Research Data.

The shoots and roots growth of the sugarcane pre-sprouted seedlings were significantly influenced ($P < 0.05$) by the inoculant rates containing *A. brasilense* applied, except the genotype RB855536 (Table 3). The greater accumulation of shoot, root, and total dry matter for the genotypes IACSP95-5000, RB855035 and RB867515 were obtained in the inoculant application rates ranging from 1.9 to 2.8 mL per seedling (Table 3).

Table 3 - Coefficients of the regression equations and determination coefficients (R^2) for the polynomial regression analyzes, optimal inoculant rate and maximum estimated value (Y_E) for the shoot, root, and total dry matter of the four sugarcane genotypes inoculated with *A. brasilense*

Genotype	Regression coefficients			R^2	*P*	Optimal inoculant rate (mL/seedling)	Maximum value (Y_E)
	Intercept (β_0)	β_1	β_2				
	Shoot dry matter (g)						
IACSP95-5000	2.52	1.28	–0.305	0.91	0.001	2.1	3.87
RB855035	2.65	1.53	–0.353	0.63	0.001	2.2	4.31
RB855536	3.43				0.397	–	–
RB867515	2.56	1.08	–0.220	0.51	0.005	2.4	3.88
	Root dry matter (g)						
IACSP95-5000	1.05	0.43	–0.116	0.76	0.037	1.9	1.45
RB855035	1.20	0.71	–0.170	0.94	0.003	2.1	1.94
RB855536	1.84				0.071	–	–
RB867515	0.94	0.52	–0.094	0.66	0.043	2.8	1.62
	Total dry matter (g)						
IACSP95-5000	3.57	1.72	–0.422	0.88	0.001	2.0	5.32
RB855035	3.85	2.25	–0.523	0.82	0.001	2.1	6.27
RB855536	5.27				0.511	–	–
RB867515	3.50	1.61	–0.314	0.57	0.010	2.6	5.57

P: Probability of the F test for the significance of the regression model.
Source: Research Data.

The maximum accumulation of shoot dry matter of pre-sprouted seedlings ranged from 3.87 to 4.34 g for genotypes IACSP95-5000 and RB855035, respectively. The maximum accumulation of root dry matter from pre-sprouted seedlings ranged from 1.45 to 1.94 g for the genotypes IACSP95-5000 and RB855035, respectively (Table 3). Gírio *et al.* (2015) also reported that the inoculation of *A. brasilense* improved the dry matter accumulation of the sugarcane seedlings. Similarly, Pereira *et al.* (2013) showed that the *A. brasilense* inoculation resulted in greater dry matter accumulation of the seedlings in some of the studied sugarcane genotypes.

The inoculant rates containing *A. brasilense* applied resulted in a linear increase in height: diameter ratio (HDR) of the genotype IACSP95-5000, whereas the smallest HDR of the genotypes RB855035 and RB 867515 was obtained with the application of 2.1 and 3.3 mL inoculant per seedling, respectively (Table 4). The shoot: root dry matter ratio was not significantly affected ($P > 0.05$) by the application rates of *A. brasilense* in any of the sugarcane genotypes (Table 1), and the average value of shoot: root ratio was 2.4 g/g.

Table 4 - Coefficients of the regression equations and determination coefficients (R^2) for the polynomial regression analyzes, optimal inoculant rate and maximum estimated value (Y_E) for the plant height: stalk diameter ratio (HDR) and Dickson quality index (DQI) of the four sugarcane genotypes inoculated with *A. brasilense*

Genotype	Regression coefficients			R^2	*P*	Optimal inoculant rate (mL/seedling)	Maximum value (Y_E)
	Intercept (β_0)	β_1	β_2				
	Height: diameter ratio (HDR)						
IACSP95-5000	8.90	0.23		0.51	0.112	–	–
RB855035	10.60	–1.04	0.243	0.76	0.003	2.1	9.49†
RB855536	9.87				0.228	–	–
RB867515	11.51	–1.22	0.185	0.80	0.023	3.3	9.50†
	Dickson quality index (DQI)						
IACSP95-5000	0.31	0.142	–0.0367	0.93	0.002	1.9	0.44
RB855035	0.30	0.214	–0.0503	0.94	0.001	2.1	0.53
RB855536	0.45				0.259	–	–
RB867515	0.25	0.152	–0.0277	0.63	0.016	2.7	0.46

† Represents the estimated minimum value. *P*: Probability of the F test for the significance of the regression model.

Source: Research Data.

The highest value of the Dickson quality index (DQI) of pre-sprouted seedlings of genotypes IACSP95-5000, RB855035, and RB867515 was obtained with the application of 1.9, 2.1, and 2.7 mL inoculant per seedling, respectively (Table 4). The maximum value of the IQD of pre-sprouted seedlings ranged from 0.44 to 0.53 g for the genotypes IACSP95-5000 and RB855035, respectively.

The use of inoculant containing *A. brasilense* improved the growth and quality of pre-sprouted seedlings of the genotypes IACSP95-5000, RB855035 and RB867515, whereas the growth and quality of pre-sprouted seedlings of the sugarcane genotype RB855536 were not significantly influenced by the application rates of inoculant containing *A. brasilense* (Tables 2, 3, and 4).

These results indicate that the higher plant growth and quality of pre-sprouted seedlings in response to inoculation with *A. brasilense* are dependent on the sugarcane genotype, confirming the evidence reported by Schultz *et al.* (2012). Similarly, Pereira *et al.* (2013) showed that the *A. brasilense* inoculation resulted in greater dry matter accumulation of the seedlings in some of the studied sugarcane genotypes. Matoso *et al*. (2020) showed that some sugarcane genotypes are more responsive to the inoculation of plant growth-promoting bacteria than other genotypes. Therefore, it is important to understand the response of sugarcane genotypes to the inoculation of plant growth-promoting bacteria (PGPB).

Greater growth and development of sugarcane plants at the inoculation of *A. brasilense* has been commonly reported in other studies . Gírio *et al*. (2015) also reported that the use of PGPB increased the plant height and shoot dry matter production of the sugarcane genotype RB867515. Schultz *et al*. (2012) concluded that the inoculation of PGPB provided an increase in the growth of RB867515 genotype plants similar to the application of 120 kg ha^{-1} of N.

The increase in stalk diameter of sugarcane plants with the PGPB inoculation was also reported in the study by Oliveira and Simões (2016). Santos *et al*. (2019) observed that the PGPB inoculation provided greater accumulation of plant dry matter in the two sugarcane genotypes (i.e., RB867515 and IACSP95-5000). Oliveira *et al*. (2018) showed that the use of PGPB resulted in a 25% increase in the accumulation of total dry matter from sugarcane plants when compared to uninoculated plants.

Pereira *et al*. (2013) also reported that the use of PGPB has the potential to improve the sugarcane seedlings growth. These inferences combined with the results presented in this study prove the physiological effect of growth-promoting bacteria, such as *A. brasilense* in improving the sugarcane plants growth. *Azospirillum brasilense*, in addition to atmospheric N_2 fixation capacity, has the potential to improve plant growth and development, especially through the synthesis of growth-promoting substances, such as auxins, gibberellins and cytokinins, and synthesis of siderophores (FUKAMI *et al*., 2018).

These bacteria can promote the shoot growth and induce the emission of new leaves on the plants (SERNA-COCK *et al*., 2011; MOURA *et al*., 2019; GONÇALVES *et al*., 2020). This increase in the number of leaves makes the plants more efficient in the use of solar radiation and, consequently, there is an increase in the photosynthesis rate and the accumulation of dry matter of the plants (OLIVEIRA *et al*., 2006).

The optimal rate of inoculant containing *A. brasilense* to be applied in transplanting sugarcane seedlings based on all the morphological traits and quality indexes of pre-sprouted seedlings can range from 2 to 3 mL of inoculant per seedling (Tables 2, 3 and 4). These results confirm the evidence previously reported by Gonçalves *et al*. (2020), which showed that the use of 4 mL per seedling of inoculant containing *A. brasilense* caused phytotoxic effects on the plant development of sugarcane genotype RB867515 up to 60 days after transplanting. This evidence shows the importance of establishing the ideal inoculant rate to be applied since high inoculant rates cause phytotoxic effects.

4 Conclusion

The use of inoculant containing *Azospirillum brasilense* improved the growth and quality of pre-sprouted seedlings of the sugarcane genotypes 'IACSP95-5000', 'RB855035', and 'RB867515'.

The optimal application rate of inoculant containing *Azospirillum brasilense* for the production of high-quality pre-sprouted sugarcane seedlings varies between 2 and 3 mL of inoculant per seedling.

The genotype 'RB855536' has no response to the application of inoculant containing *A. brasilense* during the production phase of pre- sprouted seedlings.

References

AGAMI, R.A. *et al.* Exogenous application with plant growth-promoting rhizobacteria (PGPR) or proline induces stress tolerance in basil plants (*Ocimum basilicum* L.) exposed to water stress. *Int. J. Environ. Agric. Res.*, v.2, n.5, p.78-92, 2016.

ALMEIDA-NETO, L.A. *et al*. Growth of pre-sprouted sugarcane seedlings submitted to supplementary lighting. *Rev. Bras. Eng. Agríc. Amb.*, v.24, n.3, p.194-199, 2020. doi: 10.1590/1807-1929/agriambi.v24n3p194-199

AQUINO, G.S. *et al*. Development of pre-sprouted seedlings (PSS) of sugarcane under different amounts of filter cake and application modes. *Semina: Ciênc. Agrárias*, v.9, n.5, p. 1899-1908, 2018. doi: 10.5433/1679-0359.2018v39n5p1899

BASHAN, Y.; HOLGUIN, G. Azospirillum-plant relationships: environmental and physiological advances. *Can. J. Microbiol.*, v.43, n.2, p.103-121, 1997. doi: 10.1139/m97-015

BRAGA, N.C.C. *et al*. Production of sugarcane seedlings pre-sprouted in commercial and alternative substrates with by-products of the sugarcane industry. *Semina: Ciênc. Agrárias*, v.40, n.1, p.33-48, 2019. doi: 10.5433/1679-0359.2019v40n1p33

BULEGON, L.G. *et al*. Enzymatic activity, gas exchange and production of soybean co-inoculated with *Bradyrhizobium japonicum* and *Azospirillum brasilense*. *Aust. J. Crop Scie.*, v. 11, n. 7, p. 888-896, 2017. doi: 10.21475/ajcs.17.11.07.pne575

DICKSON, A.; LEAF, A.L.; HOSNER, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forest Chronicle*, v.36, p.10-13, 1960.

FRANCO, C.B. *et al*. Qualidade de mudas pré-brotadas de cana-de-açúcar em resposta ao tamanho do tubete e do minirrebolo. *Rev. Bras. Tecnol. Agroind.*, v.14, n.1, p.3089-3103, 2020. doi: 10.3895/rbta.v14n1.10397

FUKAMI, J.; CEREZINI, P.; HUNGRIA, M. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *AMB Express*, v.8, n.73, p.1-12, 2018. doi: 10.1186/s13568-018-0608-1

GÍRIO, L.A.S. *et al*. Bactérias promotoras de crescimento e adubação nitrogenada no crescimento inicial de cana-de-açúcar proveniente de mudas pré-brotadas. *Pesq. Agropec. Bras.*, v.50, n.1, p.33-43, 2015. doi: 10.1590/S0100-204X2015000100004

GONÇALVES, M.C. *et al*. Nitrogênio e *Azospirillum brasilense* no desenvolvimento inicial da cana-de-açúcar. *Colloquium Agrariae*, v.16, n.2, p.72-81, 2020. doi: 10.5747/ca.2020.v16.n2.a360

HUNGRIA, M.; NOGUEIRA, M.A.; ARAUJO, R.S. Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *Am. J. Plant Scie.*, v.6, n.6, p.811-817, 2015. doi: 10.4236/ajps.2015.66087

INAGAKI, A.M. *et al*. Phosphorus fertilization associated to inoculation of maize with diazotrophic bacteria. *African J. Agricul. Res.*, v.9, n.48, p.3480-3487, 2014. doi: 10.5897/AJAR2014.9103

LANDELL, M.G.A. *et al*. *Sistema de multiplicação de cana-de-açúcar com uso de mudas pré-brotadas (MPB), oriundas de gemas individualizadas*. Ribeirão Preto: Instituto Agronômico de Campinas, 2012..

LOPES, V.R. *et al*. Interaction between sugarcane families and plant growth-promoting bacteria in two crop cycles. *Semina: Ciênc. Agrárias*, v.40, n.2, p.527-538, 2019. doi: 10.5433/1679-0359.2019v40n2p527

MARKS, B.B. *et al*. Biotechnological potential of rhizobial metabolites to enhance the performance of *Bradyrhizobium japonicum* and *Azospirillum brasilense* inoculants with the soybean and maize crops. *Appl. Microbiol. Biotechnol.*, v.3, n.21, p.1-10, 2013. doi: 10.1186/2191-0855-3-21

MATOSO, E.S. *et al*. Influência do uso de um mix de bactérias diazotróficas na biometria e no conteúdo de clorofila de plantas de cana-de-açúcar. *Braz. J. Develop.*, v.6, n.2, p.7261-7274, 2020. doi: 10.34117/bjdv6n2-141

MOURA, J.B. *et al*. Influence of nitrogen fixing bacteria in the establishment of pre-broken sugar cane. *Asian J. Microbiol. Biotech. Environ. Scie.,* v.21, n.1, p.22-26, 2019.

MOUTIA, J.F.Y. *et al*. Plant growth promotion by *Azospirillum* sp. in sugarcane is influenced by genotype and drought stress. *Plant and Soil*, v.337, p.233-242, 2010. doi: 10.1007/s11104-010-0519-7

OLIVEIRA, A.R.; SIMÕES, W.L. Cultivares de cana-de-açúcar inoculadas com bactérias diazotróficas em condições irrigadas no semiárido brasileiro. *Rev. Energia Agric.*, v.31, n.2, p.154-161, 2016. doi: 10.17224/EnergAgric.2016v31n2p154-161

OLIVEIRA, A.L.M. *et al*. Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. *Plant Soil*, v.284, n.1, p.23-32, 2006. doi: 10.1007/s11104-006-0025-0

OLIVEIRA, H.P. *et al*. Performance of pre-sprouted sugarcane seedlings in response to the application of humic acid and plant growth-promoting bacteria. *Semina: Ciênc. Agrárias*, v.39, n.3, p.1365-1370, 2018. doi: 10.5433/1679-0359.2018v39n3p1365

PEREIRA, W. *et al*. Acúmulo de biomassa em variedades de cana-de-açúcar inoculadas com diferentes estirpes de bactérias diazotróficas. *Rev. Ciênc. Agron.*, v.44, n.1, p.363-370, 2013.

SANTOS, F.A.; BORÉM, A. (Org.). *Cana*: do plantio a colheita. Viçosa: UFV, 2013. .

SANTOS, S.G. *et al*. Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria. *Appl. Soil Ecology*, v.133, p.12-23, 2019. doi: 10.1016/j.apsoil.2018.08.015

SCHULTZ, N. *et al*. Avaliação agronômica de variedades de cana-de-açúcar inoculadas com bactérias diazotróficas e adubadas com nitrogênio. *Pesq. Agropec. Bras.*, v.47, n.3, p.261-268, 2012. doi: 10.1590/S0100-204X2012000200015

SERNA-COCK, L.; ARIAS-GARCÍA, C.; HERNANDEZ, L.J.V. Efecto de la biofertilización sobre el crecimiento en maceta de plantas de caña de azúcar (*Saccharum officinarum*). *Biotecnol. Sector Agropec. Agroind.*, v.9, n.2, p.85-95, 2011.

SHIN, W. *et al*. Role of diazotrophic bacteria in biological nitrogen fixation and plant growth improvement. *Korean J. Soil Scie. Fertilizer*, v.49, n.1, p.17-29, 2016. doi: 10.7745/KJSSF.2016.49.1.017

SOUZA, R.; AMBROSINI, A.; PASSAGLIA, L.M.P. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics Mol. Biol.*, v.38, n.4, p.401-419, 2015. doi: 10.1590/S1415-475738420150053

TEIXEIRA, G.C.M. *et al*. Silicon in pre-sprouted sugarcane seedlings mitigates the effects of water deficit after transplanting. *J. Soil Scie. Plant Nutr.*, v.20, n.1, p.1-11, 2020. doi: 10.1007/s42729-019-00170-4

URQUIAGA, S. *et al*. Evidence from field nitrogen balance and 15^N natural abundance data for the contribution of biological N_2 fixation to Brazilian sugarcane varieties. *Plant Soil*, v.356, n.2, p.5-21, 2012. doi: 10.1007/s11104-011-1016-3