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Research Article

Diazotrophic bacteria and substrates in the growth and nitrogen accumulation of sugarcane seedlings

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ABSTRACT: Little is known about the interaction between the inoculation of diazotrophic bacteria, the variety, and the substrate used in inoculated sugarcane seedling production. Bearing this in mind, the aim of this study was to evaluate the effects of diazotrophic bacteria inoculation, four sugarcane varieties and four different substrates on the growth and nitrogen accumulation of sugarcane seedlings. Parameters related to sprouting, height, and root growth, as well as nitrogen accumulation, were evaluated. The results indicate that inoculating sugarcane seedlings belonging to the following varieties: RB867515, RB92579, RB966928 and RB975932 with bacteria may increase sprouting speed index, height, root length, fresh and dry matter weight, as well as nitrogen accumulation, which has resulted in a more uniform stem diameter. However, the responses to inoculation differ from variety to variety and are also dependent on the substrate used with better results observed in the commercial substrate and the substrate mixtures containing a higher proportion of organic compost. Understanding the interaction between the variety and the substrate with the bacterial inoculation is essential to the success of the production of inoculated sugarcane seedlings.

Keywords: *Saccharum* sp., carbonized rice husk, organic compost, biological nitrogen fixation

Introduction

Brazil is the world's largest producer of sugarcane (*Saccharum* sp.), and accounts for more than 25 % of global production, with an area under cultivation of approximately 8.7 million hectares (IBGE, 2017). One of the major problems of sugarcane cultivation is conventional planting since this practice involves the use of approximately 18 t of stems per hectare. One alternative is the utilization of pre-sprouted sugarcane seedlings, since this planting technology requires only one bud for seedling production and reduces the volume of propagating material applied by 90 % (Oliveira et al., 2018). Furthermore, since seedlings have superior growth potential and a high tillering rate, they contribute to better control of invasive vegetation, which reduces the costs of crop management (Pereira et al., 2013). In addition, it is important to select a good substrate that should provide all the conditions necessary for healthy vegetative growth, and in particular, ensure the proper development of roots, as this is a determining factor in sugarcane survival after field transplantation (Asaduzzaman et al., 2015).

The association of diazotrophic bacterial species with sugarcane is known for their beneficial effects on promoting plant growth in different ways, especially through nitrogen-fixing and the production of phytohormones and siderophores, inorganic phosphate solubilization, and improving the resistance of plants to pathogens (Spaepen et al., 2007). Studies have shown that inoculating sugarcane seedlings with associative plant-growth-promoting bacteria increases sugarcane growth and yield, and improved disease resistance (Gírio et al., 2015), as well as increasing bud sprout rate and root emission, which is beneficial to seedling production (Chaves et al., 2015).

However, little is known about the interaction between the diazotrophic bacteria inoculation, the variety, and the substrate used, which may be the key to the success of inoculated sugarcane seedling production. Therefore, this study aimed to evaluate the effects of inoculation, four sugarcane varieties and four different substrates on the rapid securing of growth and nitrogen accumulation in sugarcane seedlings, targeting a crop stand establishment and increases in plant growth and productivity.

Materials and Methods

Location and experimental design

The experiment was carried out in a protected environment in the municipality of Pelotas, Rio Grande do Sul, Brazil, 52°26′25″ W and 31°40′41″ S, at an altitude of 60 m a.s.l. The greenhouse where the work took place was made of alveolar polycarbonate, and had a gable roof measuring 12.8 m × 12 m. More antechamber access was located in front of the greenhouse, totaling 165.6 m². The on-site temperature was managed through a cooling system, ventilators, and the use of shading screens, and the temperature was maintained between 25 and 28 °C. A system of floating trays and micro-sprinklers provided irrigation.

We used a randomized complete design (RCD), with three replicates. Each experimental unit was represented by a tray containing 54 plants in individualized tubes with a volume of 180 cm³. The factors were arranged in a trifactor scheme (4 × 4 × 2), totaling 32 treatments.

Four commercial sugarcane varieties (*Saccharum* sp. hybrids) were used, namely medium-late cycle RB867515 and RB92579, and early cycle RB966928 and RB975932. As regards the diazotrophic bacteria, the treatments administered were as follows: control (without inoculation), and mixed inoculation with five strains. The substrates consisted of three combinations of carbonized rice husk (CRH) with organic compost (OC), in concentrations of 25, 50, and 75 %, respectively, as well as a commercial substrate.

Substrates

Organic compost used to prepare the substrates was produced from the composting of household waste, vegetables, cattle manure, litter swabs, rock dust, and sawdust. The maturation of the organic compost took approximately two months. The rice husk was charred and then washed, and the two materials were mixed on a volume basis, until the proportions stipulated for each of the treatments were reached (i.e. 25, 50, and 75 %). These substrates are referred to as 75CRH25OC (75 % carbonized rice husk, 25 % organic compost), 50CRH50OC (50 % carbonized rice husk, 50 % organic compost), and 25CRH75OC (25 % carbonized rice husk, 75 % organic compost). Additionally, we used a commercial substrate. Before the start of the experiment, chemical and physical analyses of the substrates were performed, and the results are shown in Table 1. Volumetric density, water retention capacity and porosity were analyzed according to the methodology described by Fermino (2014). Moisture content was identified by the standard method. The pH was determined in a 1: 5 dilution substrate solution being read at a benchtop pH and organic matter content, ash content and quantification of the macronutrients were determined following the procedure described by Abreu (2007).

Seedling production and inoculation

For the seedling production, small stalks, i.e. stem segments with individualized buds, were used, following the methodology developed in India by ICRISAT (2009). The material was taken from the median region of stems of ten month old plants. After being cut, the small stalks were cleaned, and were subjected to thermotherapy was for 30 min at 52 °C. Subsequently, treatment with systemic fungicide based on pyraclostrobin was applied for 3 min, and stalks under the inoculation treatment were then inoculated with a mixture of diazotrophic bacteria containing the following: *Gluconacetobacter diazotrophicus* BR11281^T = PAL-5^T, described by Cavalcante and Döbereiner (1988); *Herbaspirillum seropedicae* BR11335 = HRC54, described by Baldani et al. (1986); *H. rubrisubalbicans* - BR11504 = HCC103, described by Baldani et al. (1996); *Paraburkholderia tropica* - BR11366^T = PPe8^T, previously belonging to the genus *Burkholderia*, described by Reis et al. (2004) and recently reclassified by Oren and Garrity (2015); and *Nitrospirillum amazonense* - BR11145 = CBAMc, previously belonging to the genus *Azospirillum,* described by Magalhães et al. (1983) and reclassified by Lin et al. (2014). These bacteria had been previously tested and selected by Oliveira et al. (2002, 2006) with monitoring of their population during the development of sugarcane seedlings and were provided by the Diazotrophic Bacteria Collection of Embrapa Agrobiologia - CBR Johanna Döbereiner.

The inoculation medium was produced at Embrapa Agrobiologia with each bacterial strain being individually cultured in a DYGS culture medium (Baldani et al., 2014). Once the purity had been confirmed, a complete colony was inoculated in 5 mL of DYGS medium and grown in a rotary shaker at 150 rpm and 30 °C for 48 h. Subsequently, 1 mL of the culture was transferred to 75 mL DYGS medium for culturing with the same conditions for approximately 24 h until a population density of 10^8 cells mL^{-1} had been reached, which was measured using the Neubauer counting method. The inoculant counts and plant population after the tube phase were performed using the most probable number technique, as described by Baldani et al. (2014), with four semi-solid N-free media, LGI-P, JNFb, LGI, and JMV, suggested *G. diazotrophicus, Herbaspirillum sp., N. amazonense,* and *P. tropica*, respectively. After this, 175 mL of each strain was added to neutralized sterile peat to produce 250 g inoculant per strain.

A mixture of the inoculants was produced for inoculation as described by Santos et al. (2017). The five inoculum packages contained 108 bacterial cells g^{-1} peat before planting (1,250 g) diluted in 50 L of water and the small stalks were immersed in the solution for 30 min. After this procedure, the small stalks were planted in tubes with a volume of 180 cm³, containing substrate. The tubes were kept in a greenhouse for the sprouting of buds and growth of seedlings.

Table 1 – Analysis of carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium, (Mg), C:N ratio, pH, moisture (M), organic matter (OM), ashes (A), water-holding capacity (WHC), volumetric density (VD), and total porosity (TP) of substrates formulated by mixtures of carbonized rice husk (CRH) and organic compost (OC) at three concentrations (25, 50, 75 %), and a commercial substrate.

Substrate	C	N	P	K	Ca	Mg	C:N	pH	M	OM	A	WHC	VD	TP
	g kg^{-1}								%					
Commercial	268.00	6.40	0.34	2.20	2.19	1.02	42:1	6.48	40	78	22	42	63	46
75CRH25OC	267.19	3.39	0.71	3.56	1.53	0.93	79:1	5.33	47	51	49	21	43	78
50CRH50OC	263.30	5.53	0.59	3.50	5.69	1.69	63:1	6.18	39	43	57	23	47	72
25CRH75OC	259.41	7.68	0.47	3.45	9.86	2.44	47:1	5.59	27	51	49	27	53	66

Assessment of seedling characteristics and statistical analysis

After the small stalks were planted, we recorded the number of seedlings sprouted, with aerial parts formed, every three days, until the 20th day, when emergence of seedlings stabilized, to determine the emergence speed index (ESI), referred to in this study as the sprouting speed index (SSI). This was calculated using the formula proposed by Maguire (1962): SSI = E1 / N1 + E2 / N2 + ... En / Nn where: SSI = sprout velocity index; E1, E2, ... En = number of normal seedlings recorded in the first count, in the second count, and in the last count; N1, N2, ... Nn = number of days since sowing at the first, second, and last count.

At the end of 30 days, nine plants were selected at random in each treatment for seedling evaluations. The height and root length (cm) were measured with a ruler, while the stem diameter (mm) was measured using a pachymeter in the median region of the first node formed. Fresh matter (g) was determined using a precision scale (accurate to 0.01 g), and then the seedlings were packed in paper bags and taken to the greenhouse, where they remained for 24 h at a temperature of 60 °C for drying and subsequent evaluation of the dry matter. For the evaluation of total nitrogen (g), the dried plants went through a 2 mm mesh of a knife mill, and the amount of nitrogen was determined by analyzing the solid plant tissue in a Perkin-Elmer Elementary Analyzer (world standard). This technique is based on the Pregal and Dumas method, where samples are burned in a pure oxygen environment, and the gases resulting from this combustion are carried by high purity helium (an inert gas) to the detection zone (Kaiser, 1970).

A statistical analysis was conducted using the SAS (Statistical Analysis System, version 8.2). The data obtained were analyzed for normality by the Shapiro-Wilk test. Homoscedasticity was evaluated using the Hartley test, and the independence of the residuals was verified graphically. Subsequently, the data were submitted to analysis of variance (ANOVA), and in the case of statistical significance ($p \leq 0.05$ and $p \leq 0.01$), the effects of the varieties and the substrates were compared using the Tukey test, and the effects of the inoculation were compared using the t test.

Results

With the application of the F test in the ANOVA, we found significant interactions between sugarcane variety and inoculation and sugarcane variety and substrate. We also found that the responses of the different sugarcane varieties varied according to the substrate used in the production of seedlings.

In general, the sprouting speed index (Figure 1) differed between sugarcane varieties, with the highest values found in the RB975932 variety, followed by the RB966928 variety. However, inoculation with diazotrophic bacteria stimulated the SSI of both the

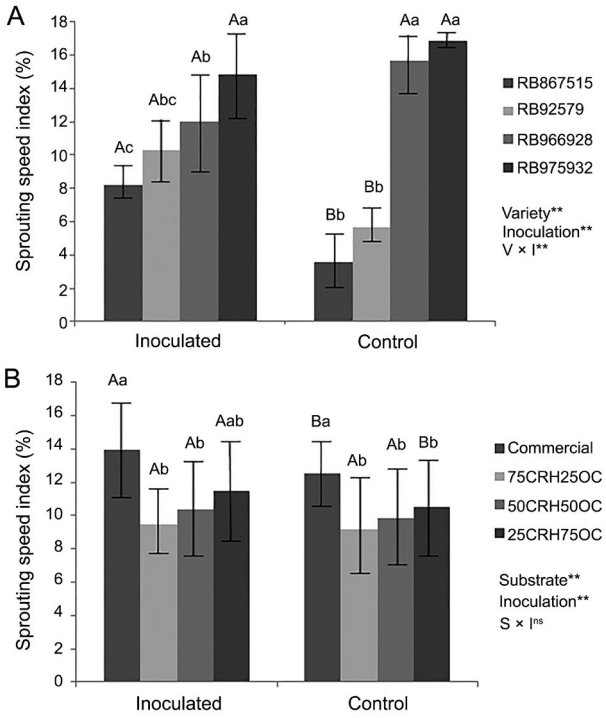

Figure 1 – Effects of the variety, substrate, and inoculation with diazotrophic bacteria on the sprouting speed index (SSI) in sugarcane seedlings. ns = Not significant; **Significant at 1 % probability. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

RB867515 and RB92579 varieties, and the SSI was approximately 50 % higher in inoculated seedlings, which reduced the sprouting difference between the four varieties tested.

With regard to the substrates, the SSI was higher when the commercial substrate was used, independent of the inoculation treatment. However, the commercial substrate, as well as the 25CRH75OC substrate resulted in significant increases in shoot bud growth when used together with the inoculation with diazotrophic bacteria treatment.

As regards the evaluation of height (Figure 2), RB92579 and RB966928 responded positively to inoculation. Additionally, the latter was superior to the other varieties. It was also observed that the growth rate of the uninoculated (control) seedlings was greater when the commercial and 25CRH75OC substrates were used. However, when plants were inoculated with diazotrophic bacteria, there was no effect of any of the

four substrates on plant height. As such, the interaction between the inoculation and the substrate led to the growth of more uniform seedlings.

The interaction between the inoculation and the substrate also resulted in greater uniformity with regard to the diameter of the stem of the seedlings (Figure 3), although this was not significant. For this parameter, there were isolated effects of the treatments, and an interaction effect between variety and inoculation. The response to inoculation was negative in quantitative terms, since the largest stem diameters were found in seedlings in the treatments without inoculation, and this was the case for all varieties and substrates tested. As regards the varieties, the largest stem diameters were recorded by RB975932, followed by RB92579 and as for the substrates, the commercial substrate, as well as the mixture containing 50 % of organic compost, resulted in larger stem diameters in the seedlings.

When evaluating the root length (Figure 4), we found that all varieties had longer roots when they were inoculated with diazotrophic bacteria, irrespective of the substrate. The interaction between substrate and variety was significant at 1 % probability. The best results were found for RB966928 and RB92579, with root lengths of approximately 32 cm, and in the 50CRH50OC and commercial substrates, where the seedlings had root lengths of 34 and 30 cm, respectively.

The total fresh matter of the seedlings (Figure 5) was higher in the inoculated varieties, except for RB975932, in which there was no difference in fresh matter between the treatments with and without inoculation. In RB867515 and RB966928, inoculation resulted in a 28 % increase in the production of fresh matter, and for RB92579 the increase was 16 %. Inoculation also resulted in increases of 33 and 30 % in the production of fresh matter of the seedlings grown in the 25CRH75OC and 75CRH25OC substrates. The results of the

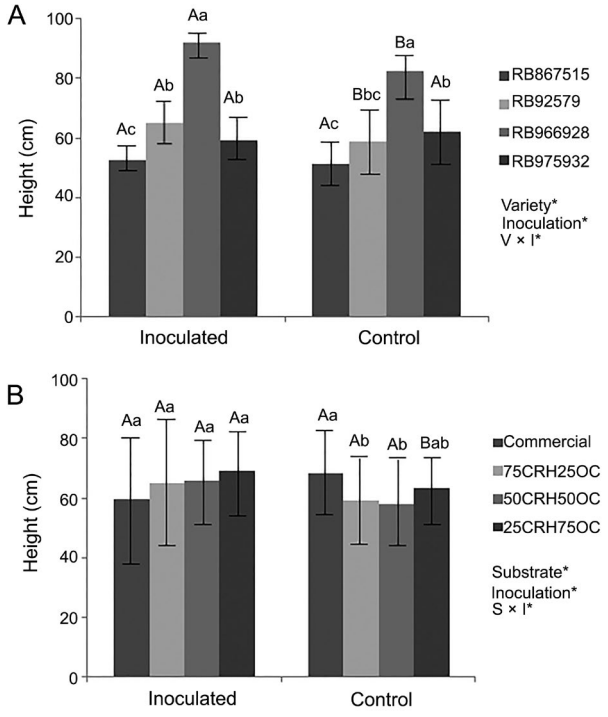

Figure 2 – Effect of the variety, substrate, and inoculation with diazotrophic bacteria on the height of sugarcane seedlings. *Significant at 5 % probability. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

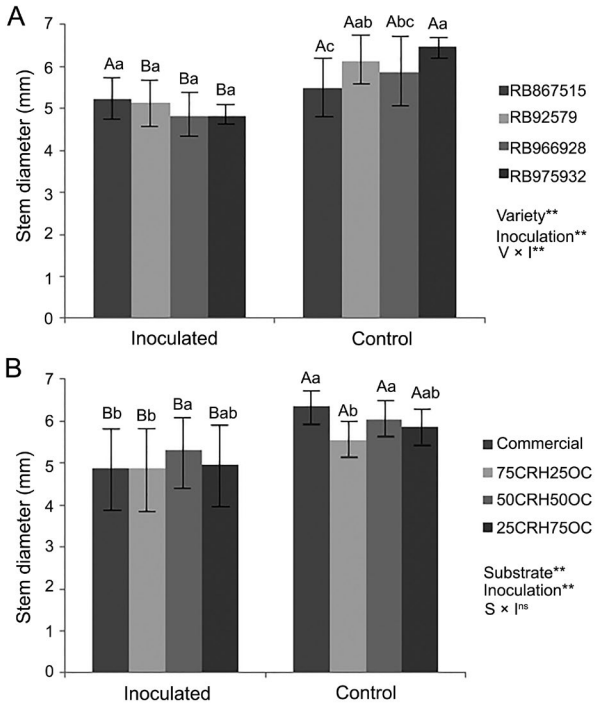

Figure 3 – Effect of the variety, substrate and inoculation with diazotrophic bacteria on the stem diameter in sugarcane seedlings. ns = Not significant; **Significant at 1 % probability. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

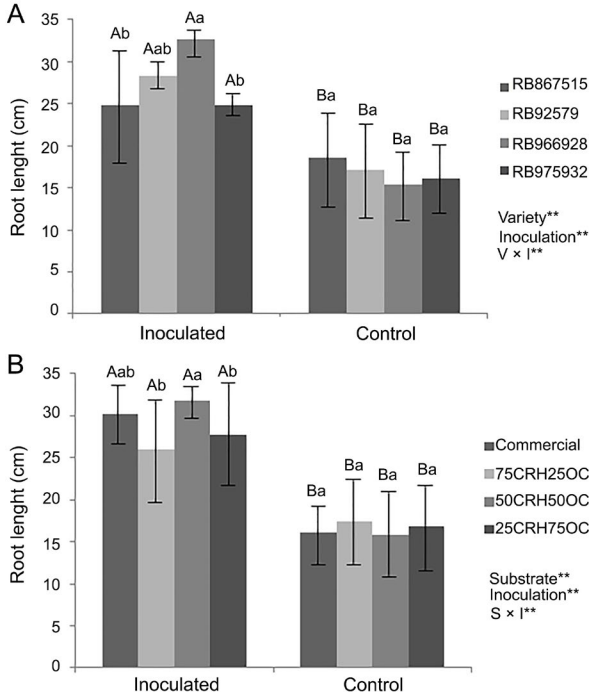

Figure 4 – Effect of the variety, substrate and inoculation with diazotrophic bacteria on the root length in sugarcane seedlings. **Significant at 1 % probability. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

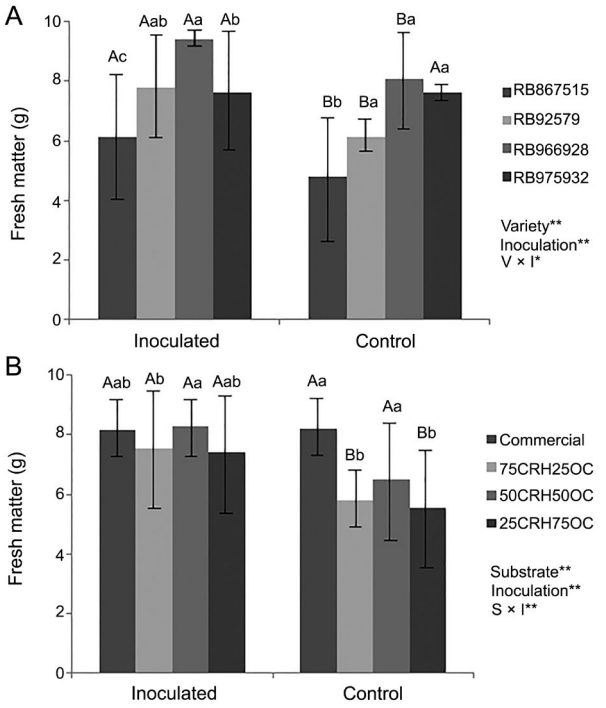

Figure 5 – Effect of the variety, substrate and inoculation with diazotrophic bacteria on the fresh matter in sugarcane seedlings. * and **Significant at 5 % and 1 % probability, respectively. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

dry matter analyses are shown in Figure 6. In the inoculation treatment, the dry weight of RB966928 was higher and did not differ from that of RB92579, but RB867515 was the only variety to respond positively to the inoculation, with an increase in dry matter of 85 % compared to the control (un-inoculated variety). As regards the substrates, the highest dry weight was found in uninoculated varieties grown in the commercial substrate. There were significant differences in dry weights of inoculated seedlings grown in the different substrates. The patterns with regard to the total nitrogen in the varieties (Figure 7) was similar, and inoculation with bacteria was beneficial only in the RB867515 variety. As regards the substrates, the commercial substrate resulted in seedlings with the highest nitrogen content, with and without inoculation with diazotrophic bacteria. However, inoculation, did not result in any significant difference between the nitrogen content of seedlings grown in the commercial substrate and those grown in the 25CRH75OC substrate.

Discussion

Differences in sugarcane responses to inoculation with nitrogen fixing bacteria were expected due to the genetic characteristics of the varieties (Oliveira, 2006). Similarly, with regard to the sprouting of the buds, which is influenced by several factors, including temperature and humidity (Compagnon et al., 2017). However, what activates the organs of the bud and of the root primordia in the sugarcane stem are changes in the nutrient reserves due to the activity of enzymes and growth regulators (Casagrande and Vasconcelos, 2010). These enzymes and growth regulators are known to be produced by the diazotrophic bacteria used in this study. This also explains the differences in plant growth between the inoculation treatments, since the production of auxins, cytokinins, gibberellins, and ethylene by these bacteria promotes plant growth (Cassán et al., 2014). Effects on the initial development of sugarcane plants have also

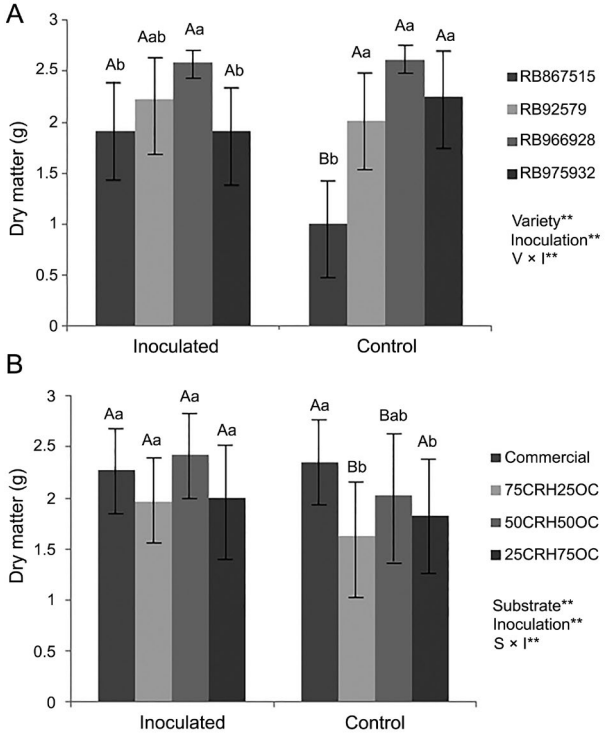

Figure 6 – Effect of the variety, substrate and inoculation with diazotrophic bacteria on the dry matter in sugarcane seedlings. **Significant at 1 % probability. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

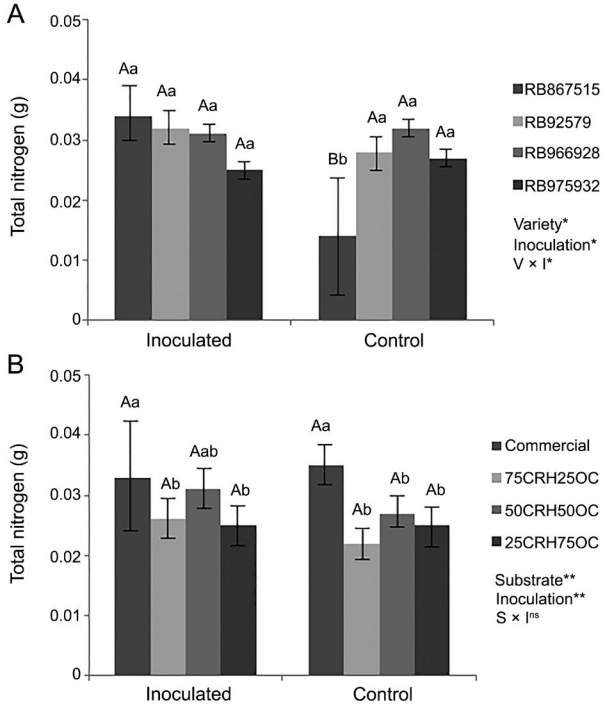

Figure 7 – Effect of the variety, substrate and inoculation with diazotrophic bacteria on the total nitrogen in sugarcane seedlings. ns Not significant. * and **Significant at 5 % and 1 % probability, respectively. Bars followed by the same capital letter do not differ from each other, when comparing the inoculation treatment to their respective control in each variety (A) or substrate (B) using the t test ($p \leq 0.05$). Similarly, bars followed by the same lowercase letter do not differ from each other, when comparing varieties (A) and substrates (B) in inoculation treatments and controls by Tukey's test ($p \leq 0.05$). 75CRH25OC: substrate composed of 75 % carbonized rice husk (CRH) + 25 % organic compost (OC); 50CRH50OC: substrate composed of 50 % CRH + 50 % OC; 25CRH75OC: substrate composed of 25 % CRH + 75 % OC.

been observed in other studies, and similar results of an increase in SSI in the RB867515 variety submitted to inoculation have been previously reported (Serna-Cock et al., 2011; Chaves et al., 2015). This change in seed sprouting speed is extremely desirable, especially in varieties of high economic importance, such as RB867515 and RB966928, which are the most commonly planted in Brazil (Gazaffi, et al., 2016).

The substrates used also influenced the sprouting of buds, since the period between sowing or planting and the emergence of seedlings is one of the critical phases of the plant cycle. During this phase, water is the main factor that influences the germination process and bud sprouting (Bradford, 1990), and should be available in appropriate abundance. We found that the highest sprouting rates, for most varieties, occurred in seedlings grown in the commercial substrate and in the mixture containing 75 % of organic compost (Figure 1). These materials have higher water retention (Table 1), promoting moisture availability for the stems during the process of sprouting the buds.

The carbonized rice husk in the mixtures is also important for bud sprouting, since it increases porosity and makes the substrate lighter, and reduces the resistance of the superficial layer to rupture by the primary sprouts (Cerqueira et al., 2015). According to Alphenaar (1993), it is important to look for porosity values between 75 and 90 %, since porosity determines the gas exchange and water movement in the container. A porosity value between 75 and 90 % is a characteristic of substrates that contain a higher concentration of CRH (Table 1).

The biometric parameters of sugarcane seedlings produced from small stalks have been studied by several authors, since it is not yet known which parameter serves as a quality indicator for the seedlings. However, in sugarcane, it is important to analyze the potential

for the accumulation of sucrose, which is related to the measurements of linear dimensions, such as height and diameter of the stem, which reflect the conditions of the root system (Sachdeva et al., 2011). As such, these parameters are important in the initial phase of seedling production.

The availability of nutrients is directly linked to the growth and development of shoot and roots, which explains the differences found between treatments with and without inoculation with diazotrophic bacteria, and between substrates. These bacteria, as previously mentioned, produce phytorials, such as auxin (Bashan and Holguin 1998; Taulé et al., 2012), which act on root growth. Increases in the length and surface of the roots of the plants also increase the absorption of water and nutrients present in the substrate (Taiz and Zeiger, 2013). This effect can be seen in Figure 4, where all inoculated varieties had a longer root length. The responses of the roots of the seedlings to the substrates also varied according to the variety of sugarcane, probably because each of the varieties had slightly different nutrient requirements, and each substrate had different nutrient availability (Table 1).

In treatments where the roots developed better, taller seedlings with higher fresh matter weights were also obtained (Figures 2 and 5). Within this scope, Oliveira et al. (2002), when inoculating sugarcane seedlings with different species of diazotrophic bacteria, isolated and mixed, observed that the combination of five species of bacteria resulted in a significant increase in the accumulation of fresh matter. Similarly, increases in shoot and root emissions were found by Figueiredo et al. (2013), in a study testing inoculation with diazotrophic bacteria and different substrates for the production of seedlings.

An additional factor is that the studies cited above emphasize that the responses of different sugarcane varieties to inoculation with nitrogen fixing bacteria are extremely variable, and when these responses are tested using different substrates, the results can vary even more. This is also supported by the results pertaining to the dry matter and total nitrogen content of the seedlings, as the only variety that responded to the inoculation with diazotrophic bacteria was RB867515. When not inoculated, this variety had the lowest accumulation of nitrogen, but when it was inoculated it had the largest accumulation of nitrogen. However, when not inoculated, variety RB867515 had the lowest dry matter content (Figure 6), and also had the shortest roots and lowest plant height. In other words, the general development of this variety was slower than that of the other varieties tested, but it was satisfactory compared with the results found by Chaves et al. (2015). The more pronounced response of RB867515 to the inoculation with bacteria was also observed in field experiments in which dry matter and total nitrogen were evaluated in sugarcane plants (Schultz et al., 2014, 2017).

The inoculation with bacteria also promoted an increase in dry matter in the seedlings grown in the 50CRH50OC and 25CRH75OC substrates (Figure 6). Figueiredo et al. (2013), however, when testing alternative substrates and a commercial one, concluded that only the latter is suitable for the production of inoculated sugarcane seedlings since the other materials are inert. This result corroborates the idea that the increase in biomass production in seedlings grown in the substrates formulated from a mixture of carbonized rice husk and organic compost may have occurred due to increases in the root lengths of seedlings induced by diazotrophic bacteria, which also increased the absorption of nutrients available in these materials. Therefore, substrates formulated from a mixture of carbonized rice husk and organic compost, as well as commercial substrate, may be suitable the production of inoculated seedlings.

This interaction between the diazotrophic bacteria and the alternative substrates is very positive under the current scenario, where it is necessary to produce more seedlings at a lower cost and with greater sustainability since this helps reduce both costs and the environmental impact of using nitrogen fertilizers.

Conclusion

Inoculating sugarcane seedlings with diazotrophic bacteria resulted in significant increases in the seedlings' growth and nitrogen accumulation in the four varieties studied. However, the responses to inoculation differed depending on the variety of sugarcane. Furthermore, the substrate used also influenced the response to inoculation and growth of sugarcane seedlings. We found that the most suitable substrates for inoculated sugarcane seedlings were the commercial substrate and the substrate mixtures containing 50 and 75 % of organic compost. Our results confirm that it is essential to understand the interaction between the variety and the substrate with the diazotrophic bacteria for the successful production of inoculated sugarcane seedlings.

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Authors' Contributions

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