SCAP
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Effect of substrates and plant growth promoting bacteria in the germination of sugarcane seeds

Efeito de substratos e bactérias promotoras do crescimento vegetal na germinação de sementes de cana-de-açúcar

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ABSTRACT

The aim of this work was to test different substrates with Plant Growth Promoting Bacteria (PGPB) inoculation on sugarcane seed germination. The substrates were sand, vermiculite and Plantmax®. The completely randomized factorial design 2x3, with 4 repetitions was used. The parameters estimated were speed of germination index, days for emergence, and 30 days after sowing the parameters: height of seedlings (cm), volume of roots (cm^3), length of roots (cm), and the number of germinated plants. The application of PGPB promoted better development of seedlings, mainly roots. The Plantmax® presented the better conditions for germination and seedling development. Vermiculite had the worst results. No response to PGPB was observed in the sand. The use of Plantmax® and PGPB in germination of sugarcane seeds is recommended.

Keywords: PGPB, vigor, *Saccharum* spp., seedling, Plantmax®

RESUMO

O objetivo do presente trabalho foi testar diferentes substratos e a inoculação com bactérias promotoras do crescimento vegetal (BPCV) na germinação de sementes de cana-de-açúcar. Os susbtratos foram areia, vermiculita e Plantmax®. O delineamento foi inteiramente casualizado em fatorial 2x3, com 4 repetições. Foram estimados os parâmetros índice de velocidade de germinação, dias para emergência, e aos 30 dias após a semeadura os parâmetros: altura das plântulas (cm), volume das raízes (cm^3), comprimento das raízes (cm) e o número de plântulas germinadas. A aplicação de BPCV promoveu o crescimento das plântulas, principalmente das raízes. O Plantmax® apresentou as melhores condições para o desenvolvimento das plântulas e para a germinação. Na vermiculita o desenvolvimento das plântulas foi limitado. Na areia não houve resposta à aplicação de BPCV. Recomenda-se a utilização do substrato Plantmax® e a aplicação de BPCV na germinação de sementes de cana-de-açúcar.

Palavras-chave: BPCV, vigor, *Saccharum* spp., plântula, Plantmax®

Introduction

The Sugarcane (*Saccharum* spp.) is one of the main crops planted in the world. Its commercial planting is through vegetative propagation, and the boundaries of its cultivation are close to the Palm Tree Line (James, 2004). The Sugarcane is widely used in the sugar and ethanol production (Matsuoka *et al.*, 2005). For the development of the culture, the breeding programs look for cultivars better adapted to the environments, with higher yields and diseases resistance (Landell and Bressiani, 2008). This process may last 13 years (Barbosa and Silveira, 2010).

The sugarcane breeding has many distinct steps, and one of the first steps is controlled crosses. The crosses are made at Sugarcane Flowering and Crossing Stations and true seeds (caryopsis) are produced (Cabral, 2007). The true or viable seed (caryopsis) is shed within the spikelet, inside of lemma and palea. If the seed is non-viable, the spikelet does not have

caryopsis inside. When many seeds are together, they are known as 'fuzz' (Cabral, 2007; James, 2004). The seeds are sent to Experimental Stations in the Breeding Programs, where will be sown according the local methodology.

Many papers about sugarcane caryopsis were produced after the 60's (Chilton *et al.*, 1965; Herbert *et al*., 1962; Silva, 1977). Cabral *et al.* (2011) and Caieiro *et al.* (2010) studied the viability and germination of crosses. Cabral (2007), observed the increase in vigor and germination percentage in 'fuzz' of sugarcane with application of gibberellic acid (GA3). Other papers with sugarcane seeds were published; however most studies were focused mainly in contamination for fungus (Cazalet and Berjak, 1983; Martins, *et al*., 2009; Sanguino and Tokeski, 1980), storage (Cabral *et al.,* 2011; Caieiro, 2008; Rao, 1982) and seed processing (Bleicher and Tokeshi, 1980; Corte Brilho and Tokeshi, 1992).

Few works have studied substrates and environments in sugarcane seed germination. Kwon-Ndung and Imolehin (2007) evaluated substrates in germination of sugarcane caryopsis and Silva *et al.* (2010) tested different environments (laboratory and greenhouse) in sowing seeds. Many works focusing substrates and germination are generally in forest seeds (Gasparin *et al.,* 2012; Martins et al., 2012), fruits (Negreiros *et al.,* 2005; Nogueira *et al*., 2013) and currently in seeds with high oil levels (Pascuali *et al*., 2012; Santos *et al*., 2013).

The use of nitrogen fertilizers in sugarcane cultivars is important to the nutrition and promotion of productivity and development of culture (Vitti *et al*., 2008). However, many studies showed that the incorrect handling of these fertilizers may pollute watercourses and atmosphere (Beaulieu *et al*., 2010; Howden *et al*., 2013; Liu *et al*., 2011). One alternative to substitute the nitrogen fertilizers, totally or partially, might be the use of inoculants of diazotrophic bacteria or Plant Growth Promoting Bacteria (PGPB) (Bashan and Holguin, 1998). These bacteria may fix nitrogen by mechanism of biological nitrogen fixation (BNF) (Boddey *et al*., 2001; Urquiaga *et al*., 1992), and/or promote the vegetal growth of plants, through production of growth regulators, phosphate solubilization and other mechanisms (Beneduzi *et al*., 2013; Lira-Cadete *et al*., 2012; Taulé *et al*., 2012). In sugarcane seeds, Madhaiyan et al. (2005) tested methylobacterial strains and verified the increase of true seed germination.

Urquiaga *et al.* (2012) estimated that cultivars of sugarcane obtain at least 40 kg N ha^{-1} yr^{-1} from association with diazotrophic bacteria. Many experiments demonstrated these bacteria are able to growth promotion and biological nitrogen fixation (BNF) in sugarcane (Silva *et al*., 2009; Silva *et al*., 2012; Pereira *et al*., 2013; Schultz *et al*., 2012). Notwithstanding these experiments, the studies with sugarcane and PGPB are often with commercial cultivars (Pereira *et al*., 2013; Schultz *et al*., 2012).

Some studies have focused on genotype-bacterium interaction in sugarcane, through BNF response from genotypes with different bacteria/strains (Caballero-Mellado and Munõz-Rojas, 2003). For this reason, breeding aiming BNF could be one way to increase its efficiency (Lopes *et al*., 2012), although it is necessary previous studies with sugarcane seeds and its viability for diazotrophic bacteria inoculation and the interactions with substrate.

These factors justify works in sense to utilize PGPB in caryopsis sowing, aiming better quality of seeds and helping breeding in future works.

The objective of this work was to test different substrates with PGPB inoculation in seeds of sugarcane.

Materials and methods

The work was performed in 'growth room' with 25 °C ± 2 of temperature, and 16 hours of photoperiod. It was utilized the sugarcane cross (family) 472B (RB931003 x RB001913), year 2009. The cross came from the Sugarcane Flowering and Crossing Station in 'Serra do Ouro' of Ridesa (Interuniversity Network for the Development of Sugarcane Industry), Alagoas State, Brazil.

For sowing, it was used 'fuzz' and the plot was represented by one pot with 1000 cm^3 capacity, with 650 cm^3 of substrate. There was 150 mg of 'fuzz' per plot. Three sterile substrates were used: two non-commercials (sand and vermiculite) and the commercial substrate Plantmax®. The label of the product shows that the commercial substrate is consisted of vermiculite, pinus bark, simple superphosphate and potassium nitrate. According to Negreiros *et al.* (2005), the chemical characteristics of the Plantmax® are: pH (H_2O) 5.47, 662.1 ppm P, 600 ppm K, 22.62 ppm Zn, 210.3 ppm Fe, 21.4 ppm Mn, 0.79 ppm Cu, 9.64 (cmolc dm^{-3}) Ca^{2+}, 3.65 (cmolc dm^{-3}) Mg^{2+} and 0.24 (cmolc dm^{-3}) Al^{3+}. The substrates were autoclaved at 1 atm, 120 °C for 60 minutes, to eliminate any microorganism that may interact with inoculant, as recommended by Brasil (2009) to the sand substrate. One sieve with 710 μm was utilized to standardize the sand. The treatments were control (non-inoculated) and inoculated with peat inoculant of diazotrophic bacteria (Table 1), of which were mixed 5 g of inoculants and 650 cm^3 of substrate. The inoculant had 10^9 bacteria g^{-1}.

To evaluate the treatments and substrates the following parameters were estimated:

Days for emergence (DE) (Edmond and Drapala, 1958) were calculated by number of days to germinate first seed.

Speed of germination index (SGI) (Maguire, 1962), using the formula:

$$SGI = \frac{\text{number of germinated seeds on 1st day}}{1} + \frac{\text{number of germinated seeds on 2nd day}}{2} + \cdots + \frac{\text{number of germinated seeds on day n}}{n}$$

Number of germinated seedlings was calculated by sum of number of germinated seeds in the plots 30 days after sowing (DAS).

Height of seedlings (cm) was determined by size of seedlings, measured with ruler 30 days after sowing DAS.

'Total plot' length (cm) and volume (cm^3) of roots – The roots were analyzed using the computer program Win/MacRhizo version 4.1c.

The completely randomized factorial design 2 x 3 (two treatments and three substrates) was used, with four replications. It was applied F-test and the treatments means were compared by Tukey test (P<0.05). Data was analyzed using the statistical software SISVAR® version 5.0, from Federal University of Lavras, Brazil (Ferreira, 2011).

Table 1 – Mix of diazotrophic bacteria and its strains.

Bacteria (Scientific name)	Strains
Azospirillum amazonense	CBAmC
Burkholderia tropica	Ppe8
Herbaspirillum seropedicae	HRC54
Herbaspirillum rubrisubalbicans	HCc103
Gluconacetobacter diazotrophicus	PAL5

Results and discussion

The analysis of variance showed significant difference to substrates in all parameters. For treatments, significant differences were detected only for speed of germination index (SGI), height of seedlings and length of roots. Significant interaction was detected for the parameters SGI, total length and volume of roots (Table 2). Altogether, the inoculant treatment was better than control, although inoculation showed negative interaction in vermiculite substrate for some parameters (Fig. 1A;B;C).

The Fig. 1A shows that for SGI in the control (not inoculated plants), there were no differences between substrates, while the application of inoculants resulted in better response in Plantmax® followed by sand. Plantmax® and sand showed positive interaction with inoculants treatment, unlike vermiculite that there was no response for the inoculants application. Studying germination of seed cane (vegetative propagation with stalks), Silva *et al*. (2012) observed better sprouting of buds when applying mixed bacteria in the sugarcane cultivar RB72454. Madhaiyan *et al.* (2005) verified the increase of sugarcane caryopsis germination with inoculation of methylobacterial strains. The authors also demonstrated in leaves of clone Co86032 higher cytokinin contents.

The behavior of substrates was different for days for emergence (DE). The sand provided the conditions for seeds to germinate first. Plantmax® had the intermediate value and vermiculite was the last one (Fig. 1D). Silva *et al.* (2010) also worked with SGI and DE evaluating sugarcane caryopsis germination and reported significant differences in both variables to different conditions of temperature.

It was observed better development in the aerial part of seedling ('factor substrate') in sand substrate, followed respectively by Vermiculite and Plantmax®

Table 2 – Variance analysis (F-test) of treatments (control and inoculated), substrates (sand, vermiculite and Plantmax®) and interaction with the respective coefficients of variation (CV) and average squares for the parameters: speed of germination index (SGI), days for emergence (DE), height of seedlings (HS), number of plants germinated (NG), total length of roots (LR) and total volume of roots (VR).

Source of variation	Average squares					
	SGI	DE	HS	NG	LR	VR
Substrates	7.20**	1.125**	37.14**	107.04**	252324.85**	0.00843**
Treatments	1.33*	0.041$^{n.s.}$	8.42*	20.17$^{n.s.}$	5988.83**	0.00002$^{n.s.}$
Interaction	2.78**	0.042$^{n.s.}$	0.50$^{n.s.}$	13.54$^{n.s.}$	58120.37**	0.00109*
CV (%)	**11.00**	**12.59**	**16.34**	**12.99**	**2.90**	**18.05**

$^{n.s.}$ Non-significant ** Significant at 1% * Significant at 5%

(Fig. 1E). Opposite to this result, in the commercial substrate a greater number of plants germinated after 30 days, followed respectively by vermiculite and sand (Fig. 1F). This result may be related the competition of plants in the plots, with Plantmax® having more competition than the others substrates. In the sand, where the seedlings were taller, less competition may have occurred due to the low number of plants germinated.

Utilizing substrates Plantmax® and sand, Martins et al. (2012) did not find difference on height of seedlings of *Schizolobium parahyba*. Until 14 DAS the seedlings in the commercial substrate were highest than sand and pine sawdust. According to the work of Negreiros *et al.* (2005), the Plantmax® presented minor values of height of seedlings in papaya tree when compared with other substrates.

Plantmax® was the best substrate for length of roots comparing other substrates in both treatments, the sand exhibited lower length of roots in control treatment, and when inoculated sand presented the intermediate value. Vermiculite was intermediate in control treatment and the last one when inoculated (Fig. 1B). To the 'factor' treatment, no difference was found on sand, the best treatment in the vermiculite was control and in the Plantmax® the inoculated treatment. The volume of roots (Fig. 1C) has followed the same tendency, excepted the substrate vermiculite that obtained the minor values to the factor substrates. Vermiculite interacted negatively and Plantmax® interacted positively in both parameters with the inoculant (Fig. 1B,C).

Probably, the higher amount of nutrients and better physical conditions in Plantmax® may influence the results of this work. Negreiros *et al.* (2005) working with papaya tree seedlings, observed the greater length of roots with the same substrate. Smirdele *et al.* (2001) have demonstrated the good conditions to germination of the Plantamax® substrate, where the accumulation of roots dry mass and seedlings were better than mixed substrates, in lettuce, cucumber and red pepper. Diniz *et al.* (2006) also verified benefits in tomato, pepper and lettuce seedlings. Catunda *et al.* (2008) have concluded that the physical characteristics of the commercial substrate, as higher retention of water, low density and higher aeration increases the averages of dry mass of the roots.

Know-Ndung and Imolehin (2007) verified better response of vermiculite in the germination of sugarcane caryopsis, however the cost of the substrate may be expensive. In this way, they justified the mixture of different substrates and vermiculite.

The presence of PGPB in inoculated treatments may produce growth regulators that implying the better development of roots and may be help on the growth of aerial part of plant as on the present work (Fig. 1E). One of those growth regulators produced by bacteria is the auxin (Taulé *et al.,* 2011), acting in the growth of roots, enlarging the absorption area, and increasing the contribution of nutrients presents in soils (Taiz and Zeiger, 2010). That effect may be related with better length and volume roots of the inoculated commercial substrate (Fig. 1B,C). Working with lettuce, Schlindwein *et al.* (2008) verified better germination and roots length of the inoculate treatments with bacteria that produce IAA.

The authors Bashan and Holguin (1997) also reported benefits of PGPB inoculation on root growth and attributed through production of IAA (indol acetic acid) of *Azospirillum* genus. The *A. amazonense* strain CBAmC, one of the bacteria that compound the bacteria mix (Table 1), demonstrated its potential to IAA production by Reis Junior *et al.* (2004), this growth regulator may be associated to the better development of roots in inoculated treatment. On the other hand Taulé et al. (2011) did not find IAA production to the same bacteria, but they have found IAA production in *Gluconacetobacter diazotrophicus* and *Herbaspirillum seropedicae*, and phosphate solubilization in *Burkholderia tropica*, all of them are also part of the bacteria mix.

Conclusions

The application of the inoculant based on PGPB demonstrated its potential to apply in sugarcane caryopsis. Among the substrates, the Plantmax® demonstrated better condition to development of caryopsis and great interaction. Generally the growth of seeds in sand has been good, however it did not presented conditions to development of caryopsis combined with bacteria. The vermiculite was not good for the development of seeds in both treatments. According the results, Plantmax® is recommended and vermiculite is not recommended to use in studies with sugarcane seeds.

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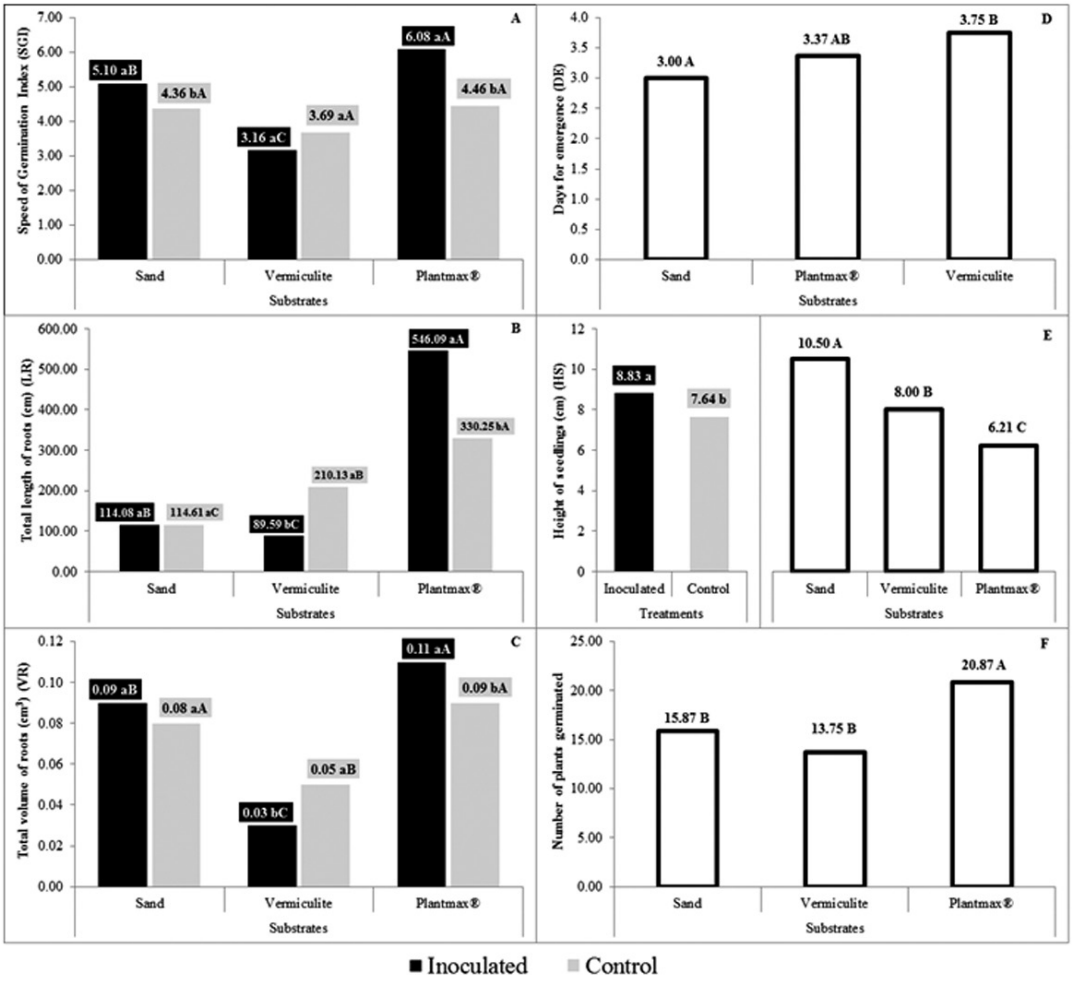

Figure 1 – Parameters estimated Speed of Germination Index (A), Total length of roots (cm) (B), Total volume of roots (cm³) (C), Days for emergence (D), Height of seedlings (cm) (E) and Number of plants germinated (F) into two treatments (Control and Inoculated) and three different substrates (Sand, Vermiculite and Plantmax®).
Means followed by the same letter in lower case for Treatments and upper case for Substrates are not significantly different at P<0.05 (Tukey's test)

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