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The Selection of Sugarcane Families That Display Better Associations with Plant Growth Promotimg Rhizobacteria

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Abstract: The capacity of the sugarcane plant to respond to Plant Growth Promoting Rhizobacteria (PGPR) is associated with both the efficiency of the bacterial strain and the capacity of the plant to respond to inoculation. For this reason, the appropriate selection of both the bacterial strain and the sugarcane genotype is required for generating optimal results from PGPR inoculations. To address this issue, this study sought to evaluate the response of 54 sugarcane families to inoculation with *Azospirillum brasilense* strains. In particular, four months after germination, 54 families from crosses between clones of sugarcane were treated either with an inoculant named Triazo, which was composed of a mixture of the Abv5, Abv6 and Abv7 strains of *A. brasilense*, or with the IC26 strain of *A. brasilense*. The treated plants were then planted in fields. These plants were assessed 14 months after they had been planted on the basis of various productivity parameters. Significant differences among the inoculants were observed for stalk length, stalk diameter and Brix. Significant interactions between the families and bacteria occurred with respect to stalk diameter and Brix; the interaction coefficients could have either positive (0.7272 for Brix and 0.4061 for stalk diameter) or negative (-0.5514 for Brix and -0.1858 for stalk diameter) values, depending on the family and the inoculant that were considered. Therefore, the inoculation of the seedling in the first phase of selection is recommended for a sugarcane breeding program that seeks to select genotypes with better responses to PGPR inoculation.

Key words: Plant-bacteria interaction, PGPB, plant breeding, *Azospirillum brasilense*, nitrogen-fixing bacteria

INTRODUCTION

The genus *Azospirillum* contains the most extensively studied Plant Growth Promoting Rhizobacteria (PGPR), which are known to enhance the yield of different crop plants (Bashan and Holguin, 1997). The *Azospirillum brasilense* species is considered to be a rhizobacterium (Broek *et al*., 1993; Assmus *et al*., 1995) that engages in associative symbiosis with various host plants (Bashan *et al*., 2004). The species was first described as *Spirillum lipoferum* (Dobereiner and Day, 1976) and was subsequently reclassified as *Azospirillum brasilense* in 1978 (Tarrand *et al*., 1978); since 1978, it has been studied for its capacity to fix nitrogen (Bashan and Bashan, 2011).

In addition to nitrogen fixation, these bacteria may also provide other beneficial biological effects to plants (Bashan *et al*., 1990; Verma *et al*., 2010), such as increasing phytohormone activity, promoting root system proliferation, enhancing the water and mineral uptake of the plant, solubilizing phosphates and mobilizing minerals. Recently, other mechanisms have also been discovered and attributed to this bacterium, including the sorting of small molecules and enzymes, the enhancement of membrane activity and proton efflux, the direct and indirect biological control of numerous phytopathogens and the mitigation of environmental stressors of plants, such as salt (Bashan and Bashan, 2011) and drought stress (Arzanesh *et al*., 2009).

For these reasons, research has been conducted that seeks to select more effective strains of Plant Growth Promoting Bacteria (PGPB) (Schloter and Hartmann, 1998; Shaukat *et al*., 2006; Hungria *et al*., 2010; Keyeo *et al*., 2011). However, the results of this research demonstrated that the capacity of PGPB strain to fix nitrogen and promote plant development is variable

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(Bashan and Levanony, 1990) and certain results emphasize that the necessity to select not only the bacteria strain but also the plant genotype (Munos-Rojas and Caballero-Mellado, 2003; De Oliveira *et al*., 2006).

The interactions between plants and bacteria depend on the plant genotype, the species and strain of bacteria that are involved, the presence of other microorganisms and the environmental conditions (Baldani and Baldani, 2005; Oliveira *et al*., 2009). Various experiments have emphasized that the plant genotype exerts an important effect on the plant-bacteria interaction. However, few investigations have been conducted with the aim of selecting gramineous plant genotypes that respond well to inoculation with PGPB, although, this topic has been addressed in the context of maize (De Mendonca *et al*., 2006), wheat (Sala *et al*., 2007) and rice (Ladha *et al*., 1987).

At present, PGPB-related studies of sugarcane are focused on evaluating the responses of commercial varieties to inoculation with PGPB (Coelho *et al*., 2003; Hari and Srinivasan, 2005). The selection of genotypes that have positive interactions with the bacteria is not a primary objective of sugarcane breeding programs.

However, the acquisition of knowledge regarding the different responses of sugarcane families to inoculation with PGPR during the initial phase of sugarcane breeding, which features greater genetic variability, can facilitate an understanding of the nature and magnitude of these interactions. This understanding can help researchers structure future studies and breeders plan new strategies of selection. Thus, the purpose of this study was to assess the responses of different sugarcane crosses (families) to inoculation with *A. brasilense* in the initial phase of a sugarcane breeding program, thereby allowing for the selection of the most responsive families.

MATERIALS AND METHODS

Site description and field management: The experiment was conducted at the Paranavai Experimental Station of the Federal University of Parana (UFPR), in the city of Paranavai/PR, Brazil (23°05'S, 52°27'W, altitude 503 m), during 2009 and 2010. According to Köppen's classification system, the climate type at the experimental station is Cfa (IAPAR 1994). The soil in the experimental station is classified as a Ferralsol (Dystric) (FAO, 2006). The main chemical characteristics of the soil at the start of the experiment are illustrated in Table 1.

Plant material: This study used seeds (caryopses) from 53 biparental crosses and one polycross between sugarcane clones; these crosses were performed in the "Serra do Ouro" Crossing Station in 2008 (Table 2). The seeds were germinated in November 2008 in a greenhouse with controlled irrigation and temperature (30°C±2), using plastic trays containing Plantmax® as a substrate.

At 30 days after sowing, the seedlings were transplanted to individual cells (60 cm^3) in Styrofoam trays which were left in the shade (50%). At four months after sowing, the plants were first treated with *A. brasilense* inoculants, then left in the shade (50%) for seven additional days and subsequently transplanted to the field (the first phase of a sugarcane breeding program).

Table 1: The chemical properties of the soils (0-40 cm) used in this experiment

pH ($CaCl_2$)	Al cmol$_c$	H+Al (dm^{-3})	Ca	Mg	K	CEC	T_{CEC}	BS (%)	N	C (g dm^{-3})	P
6.45	0.00	2.025	0.65	0.95	0.02	3.59	1.58	43.57	0.10	11.97	10.72

CEC: [a]Cation exchange capacity = H+Al+Ca+Mg+K, Total CEC: T_{CEC} = Ca+Mg+K BS: Base saturation = (T_{cec}/CEC)×100

Table 2: The relationships of the parents and crosses (female×male) that produced the 54 sugarcane families studied in this experiment

Family	Cross (female×male)	Family	Cross (female×male)	Family	Cross (female×male)
1	H64-1881×RB92579	19	RB99386×SP79-2313	37	RB867515×RB977619
2	SP83-5073×RB92579	20	RB813804×RB72910	38	RB855511×RB92606
3	RB951560×RB867515	21	RB977625×RB008004	39	RB966928×RB935845
4	RB01616×H64-1881	22	RB040826×RB855035	40	SP83-2847×RB855546
5	RB99386×SP91-1049	23	RB855002×RB93509	41	RB855536×RB925268
6	RB001922×RB92579	24	RB966922×polycross	42	RB855063×SP77-5181
7	RB93509×RB845257	25	RB961012×RB931011	43	RB855113×SP77-5181
8	RB977662×RB92579	26	RB011579×RB991555	44	RB925345×RB935686
9	RB845210×RB931003	27	RB977619×RB867515	45	RB72454×RB951019
10	RB011579×RB92579	28	SP80-1842×RB98710	46	SP77-5181×RB855113
11	RB832847×RB845210	29	RB99361×RB98710	47	RB845210×SP83-2847
12	RB956911×RB93509	30	RB931003×RB855336	48	SP85-3877×RB931565
13	RB021698×RB977625	31	H69-4234×RB01616	49	RB955114×RB845197
14	RB925378×SP80-185	32	RB01616×H69-4234	50	RB855546×RB925276
15	RB008309×RB974115	33	RB040811×RB845197	51	H64-1881×RB01616
16	RB99382×RB98710	34	RB951541×RB947520	52	RB99386×SP79-2313
17	RB69758×SP93-1322	35	RB965505×SP70-1143	53	RB92579×H64-1881
18	RB98347×RB98710	36	RB92606×H56-6724	54	RB971765×RB93509

Strains of bacteria: The *A. brasilense* strains Abv5, Abv6, Abv7 and IC26 were supplied by the UFPR Department of Biochemistry and Molecular Biology, Curitiba, Parana. The Abv5, Abv6 and Abv7 strains were isolated from maize (Hungria *et al*., 2010), whereas the IC26 strain is a natural mutant with constitutive nitrogenase activity. All four of these strains are diazotrophs. The Abv5 and Abv6 strains produce indoleacetic acid (IAA) and are capable of reducing acetylene to ethylene *in vitro* (Pedrinho *et al*., 2010).

Inoculants: The *A. brasilense* strains were cultivated separately in NFb media (4.0 g L^{-1} KH_2PO_4, 6.0 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} NaCl, 0.2 g L^{-1} $CaCl_2$, 0.056 g L^{-1} nitrilotriacetic acid, 0.2 g L^{-1} $FeSO_4.7H_2O$, 1.0×10^{-4} g L^{-1} biotin, 0.002 g L^{-1} $Na_2MoO_4.2H_2O$, 0.00235 g L^{-1} $MnSO_4.H_2O$, 0.0028 g L^{-1} H_3BO_3, 8.0×10^{-5} g L^{-1} $CuSO_4.5H_2O$ and 2.4×10^{-4} g L^{-1} $ZnSO_4.7H_2O$) at 30°C (optical density: 1.5-1.8) for 48 h. In total, 5 mmol L^{-1} glutamate was used as a nitrogen source and 0.5% of sodium lactate was used as a carbon source. After being autoclaved, these two reagents were added to cold medium and the pH of this medium was adjusted to 6.8. After the bacterial strains had grown, they were added to polyvinyl pyrrolidone (PVP) solution at 5%. The strains Abv5, Abv6 and Abv7 were mixed to form one inoculant named Triazo. The IC26 strain was used separately and constituted another inoculant for this experiment.

Inoculation: Plants from each family were subjected to one of the following treatments: no inoculation (T0); inoculation with the IC26 strain of *A. brasilense* (1×10^{10} bacteria mL^{-1}) (T1); or inoculation with Triazo (3×10^{9} bacteria mL^{-1}) (T2).

The inoculations were performed four months after sowing; at this time, the plants were still in trays. A total of 100 μL of the appropriate inoculant (at the aforementioned concentrations) was applied to the base of each plant with a micropipette.

Experimental design and planting: The experiment had a completely randomized block design with three replicates and a split-plot arrangement of treatments. Each block consisted of 54 parcels and one family was planted in each parcel. A parcel consisted of three rows, each of which was 5 m in length and contained 10 plants; each row was subjected to a different inoculation treatment (T0, T1 or T2). The space between lines was 1.4 m between rows and 0.5 m between plants and the total experimental area was 5000 m^2. The RB986419 sugarcane cultivar was used as a side border for this area.

The planting was conducted in February 2009. Fertilization was conducted in the furrows; in particular, each row (5 m in length) received 540 g of potassium chloride and 180 g of ordinary superphosphate but no additional nitrogen. In accordance with the standard procedures for sugarcane cultivation, the plants were treated with pre-emergence herbicides and post-emergence weeding but were not irrigated.

Evaluation: The evaluation of the plants was conducted 14 months after planting and the following parameters were evaluated: the number of stalks per plant, the average length of the stalk (the number of meters between the first leaf with clearly visible dewlap and the bottom of the stalk), the average diameter of the stalk (at the third internode from the bottom) and Brix.

Statistical analysis: The data for all of the evaluated parameters were analyzed using the Selegen REML/BLUP software package (De Resende, 2006) to determine statistically significant differences among the families, the bacterial treatments and the interactions between the bacteria and the plants. This software estimated the components of variance and the prediction of genetic values using the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) procedure. The statistical model used is provided below:

$$y = Xa + Zb + Wp + Qr + T(axb) + e,$$

where, y is the data vector, a is the vector representing factor A effects (assumed as fix, allocated to the plots) added to general mean; b is the genotypic effect vector associated with the sub-plot in question (representing the magnitude of factor B within the context of factor A; this magnitude is assumed to be random); p is the parcel effect vector, that is, the error associated with the interaction of factor A with the replication factor (this interaction is assumed to be random); r is the block effect vector or replication factor (assumed to be random); a x b is the vector representing the genotype×factor A interaction (also random); and e is the residual vector or error vector (random, once again). The capital letters (X, Z, W, T and S) represent the incidence matrices for referred effects (De Resende, 2006).

RESULTS

The results of the deviance analysis, obtained using the REML/BLUP procedure, are similar to the variance analyses. However, the former methodology enables better accuracy because it predicts genotypic values

Table 3: The results of the deviance analysis for the following characteristics: The number of stalks (NSP), the average length of the stalk (ALS), the average diameter of the stalk (AD) and the average Brix after 14 months

	Characteristics (F value)			
Effects	NS	ALS	AD	Brix
Family	28.30***	9.04***	13.17***	41.89***
Treatment	0.00^{ns}	16.96***	9.07***	30.77***
Block	6.54**	53.51***	24.77***	17.21***
Interaction family×treatment	2.11^{ns}	2.09^{ns}	2.97*	25.92***
CV%	18.88	7.79	6.48	4.37

***Significance at the 1% level (6.63), **Significance at the 5% level (3.84), *Significance at the 10% level (2.71), ns: Non significant

Table 4: The results of the treatments for the following characteristics: The number of stalks (NS), the average length of the stalk (ALS), the average diameter of the stalk (AD) and the average Brix after 14 months

	Average of each characteristic			
Treatment	NS^{ns}	ALS (m)***	AD (cm)***	Brix***
Non-inoculated	8.942	2.279	2.309	19.119
Triazo	8.705	2.285	2.332	18.954
IC26 strain	9.037	2.298	2.340	19.208

***Significance at the 1% level (6.63), ns: Non significant

instead of phenotypic values, thereby decreasing the impact of environmental factors on the results. The deviance analysis allows for the analysis of experiments that are unbalanced due to lost parcels or repetition. For this reason, the deviance analysis is recommended for sugarcane breeding assessments, particularly for family studies conducted in the initial phases of selection.

The results of the deviance analysis (Table 3) demonstrated that there were significant differences among the families for all of the parameters at a 1% significance level, indicating the high genetic variability present in this selection phase of a sugarcane breeding program.

The distinct bacterial treatments examined in this study demonstrated significantly different effects (at a 1% significance level) for the parameters of stalk length, stalk diameter and Brix (Table 4). In particular, the plants treated with IC26 possessed the greatest diameter (2.340 cm), whereas the plants treated with Triazo had greater diameters (2.332 cm) than the control plants (2.309 cm). A somewhat contrasting result was observed for the Brix variable, as greater values of Brix were presented by the plants that were not inoculated (19.119) than by the plants that received the Triazo treatment (18.954); however, the plants treated with IC26 produced the highest value of Brix (19.208) (Table 4). The plants inoculated with IC26 also had the longest stalks (2.298 cm); the stalk length was greater in the plants treated with Triazo (2.285 cm) than in the control (2.279 cm).

These results indicated that except for the negative effect produced by the Triazo inoculant on Brix, the inoculation of the sugarcane with *A. brasilense* positively influenced all of the studied parameters.

Although, the blocks were randomized, there was a significant difference in the block effect for all of the analyzed variables, indicating that the plant growth was influenced by the soil composition and the light that was received by the plants. Given that the soil used in field experiments is not homogeneous, this result was an expected outcome; moreover, the low values of the coefficient of variation for this block effect indicated that the experimental precision of this study was adequate.

The interaction effects for the examined sugarcane families differed significantly for the parameters of Brix (at a 1% significance level) and average stalk diameter (at a 10% significance level), indicating that the response to inoculation varied with respect to the sugarcane family that was tested (Table 6). However, the other parameters that were examined did not present significant differences due to this interaction effect.

The 10 best-performing families for each assessed parameter are illustrated in Table 5; unsurprisingly, the order of the studied families differs depending on the variable that is being evaluated. The plants of families 43 (RB855113×SP77-5181), 50 (RB855546×RB925276), 30 (RB931003×RB855336), 6 (RB001922×RB92579), 8 (RB977662×RB92579) and 2 (SP83-5073×RB92579) were among the best for more than one parameter.

In Fig. 1a, the averages of the Brix parameter for each treatment are illustrated. As this graphic demonstrates, the average of this trait changed for all treatments in accordance with the family that was examined, indicating that inoculation can promote either negative or positive family-dependent effects on productivity. For instance, the control samples of family 6 (RB001622×RB92579) presented the highest Brix values, whereas lower values of Brix were observed in the samples that were inoculated with Triazo and IC26. By contrast, the control samples of Brix were observed in the samples that were inoculated with Triazo and IC26. By contrast, the control samples of

Table 5: The genotypic value (GV) of the 10 best families with respect to the following characteristics: The number of stalks (NS), the average length of the stalk (ALS), the average stalk diameter (AD) and the average Brix at 14 months after planting

	Characteristics evaluated after 14 months							
	NS		ALS		AD		Brix	
Order	Family	GV[1]	Family	GV	Family	GV	Family	GV
1	30	12.485[a2]	20	2.437[a]	43	2.482[a]	3	20.671[a]
2	43	11.186[a]	27	2.412[a]	8	2.466[a]	2	20.658[a]
3	46	10.983[a]	11	2.401[a]	38	2.458[a]	6	20.466[a]
4	31	10.964[a]	6	2.386[a]	32	2.436[a]	12	20.298[a]
5	16	10.840[a]	25	2.383[a]	50	2.435[a]	5	20.219[a]
6	45	10.626[b]	17	2.382[a]	30	2.422[a]	24	20.100[a]
7	44	10.563[b]	43	2.373[a]	2	2.420[a]	26	20.059[a]
8	47	10.319[b]	10	2.369[a]	4	2.412[a]	37	20.027[a]
9	50	10.247[b]	8	2.357[a]	54	2.410[a]	42	20.011[a]
10	41	9.939[b]	50	2.355[a]	52	2.397[a]	14	19.989[a]

Using student's t-test, numbers followed by the same letter in each column do not differ statistically at the 1% significance level

Table 6: Summary of the ten best families (with respect to genotypic value) for the characteristics of average Brix and average stalk diameter, as well as the corresponding value of family-treatment (F×T) interactions, at 14 months after planting

	Average stalk diameter*		Brix***	
Order	F×T	Interaction value	F×T	Interaction value
1	43×T0	-0.0034	1×T0	0.3819
1	43×IC26	0.0254	1×IC26	0.0699
1	43×TRIAZO	0.0251	1×TRIAZO	-0.5514
2	8×T0	0.4061	2×T0	0.213
2	8×TRIAZO	-0.1858	2×IC26	0.0897
2	8×IC26	0.0047	2×TRIAZO	0.1451
3	38×T0	0.0249	6×T0	0.7272
3	38×IC26	0.045	6×IC26	-0.2932
3	38×TRIAZO	-0.0301	6×TRIAZO	-0.0413
4	32×T0	0.0061	4×T0	-0.5045
4	32×IC26	0.0324	4×IC26	0.4341
4	32×TRIAZO	-0.0054	4×TRIAZO	0.073
5	50×T0	0.0224	5×T0	-0.1036
5	50×IC26	0.0225	5×IC26	0.1383
5	50×TRIAZO	-0.0121	5×TRIAZO	0.2874
6	30×T0	0.0059	24×T0	0.4156
6	30×IC26	-0.0021	24×IC26	-0.4265
6	30×TRIAZO	0.025	24×TRIAZO	0.2991
7	2×T0	0.013	26×T0	0.1798
7	2×IC26	0.0056	26×IC26	0.1862
7	2×TRIAZO	0.0206	26×TRIAZO	-0.0898
8	40×T0	0.0056	37×T0	-0.4287
8	40× IC26	-0.0042	37×IC26	0.6071
8	40×TRIAZO	0.0054	37×TRIAZO	0.0886
9	9×T0	-0.0209	9×T0	-0.0968
9	9×IC26	0.0239	9×IC26	-0.0461
9	9×TRIAZO	-0.0229	9×TRIAZO	0.3047
10	52×T0	0.0119	10×T0	-0.0146
10	52×IC26	-0.0118	10×IC26	0.289
10	52×TRIAZO	0.0211	10×TRIAZO	-0.202

***Significant at the 1% level (6.63), *Significant at the 10% level (2.71)

family 37 (RB867515×RB977619) presented the lowest Brix values, whereas higher values of Brix were observed in the samples that were inoculated with Triazo and IC26.

The general trend discussed above for the Brix values of the plants of families 6 and 37 was also observed for average stalk diameters (Fig. 1b), as the changes in stalk diameter due to inoculation also varied based on the family that was examined. Another example of this variance was the contrast between the plants of family 44 (RB925345×RB935686) and the plants of family 5 (RB99386×SP91-1049); for the plants of the former family, inoculation with Triazo produced greater average stalk diameters relative to the non-inoculated controls, whereas the non-inoculated control plants of the latter family displayed the thickest average stalks. These results indicated the importance of inoculating the plant during the initial phases of a sugarcane breeding program.

Treatment×family interaction: Table 6 lists the 10 best families with respect to the genotypic values and the

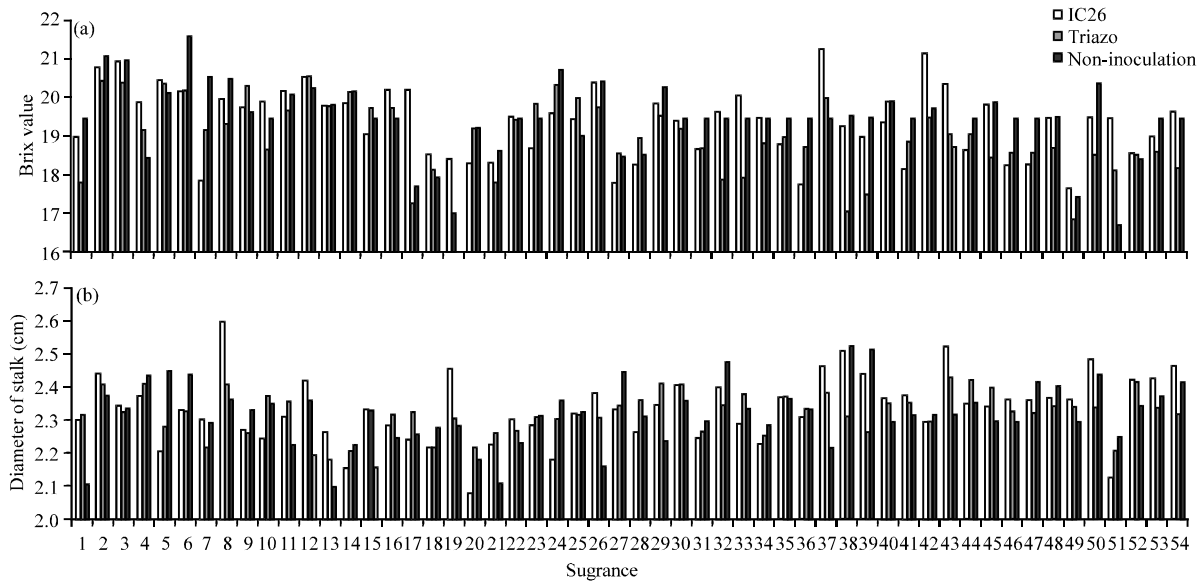

Fig. 1(a-b): (a) The genotypic value of Brix and (b) average stalk diameter of 54 families of sugarcane for each treatment (non-inoculated, inoculated with triazo and inoculated with IC26) after 14 months

values of family×treatment interactions. These values varied among the examined families and both negative and positive interaction values were observed for the same family, depending on the parameter that was evaluated.

One example is family 1 (H64-1881×RB92579), which presented a positive interaction with the Brix variable for control plants (0.382) but a negative interaction with the same variable in the plants that were inoculated with Triazo (-0.5514). A similar response was observed in family 6 (RB001922×RB92579), as the interaction with Brix was positive in the control plants (0.727) but negative in the plants that were treated with IC26 (-0.293).

Although, this selection phase presents a great number of genotypes and high genetic variability, it is possible to observe specific interactions between a family and an *A. brasilense* inoculant. For the same family, different *A. brasilense* inoculants may produce either positive or negative interactions from inoculations. For instance, the Brix values of the plants of family 24 (RB966922×polycross) were positively affected by inoculation with Triazo (0.299) and negatively impacted by inoculation with IC26 (-0.426), indicating that in this case, the response could vary based on the inoculant that was used. For this reason, studies with different inoculants are suggested.

DISCUSSION

Genetic differences among families: The significant differences observed among families for all of the evaluated parameters were expected. During the first selection phase of sugarcane breeding, the genetic variability of the population is high and this high variability indicates that conditions are favorable for selection (Ferreira *et al*., 2005; Lopes *et al*., 2008).

The effect of inoculation on plant development: We observed that inoculation produced significant differences in the traits of Brix, the average stalk length and the average stalk diameter. These results are consistent with the findings of Biari *et al*. (2008), who reported that maize responds differently to inoculation with PGPB depending on the characteristics that are evaluated. Ovando-Medina *et al*. (2007) observed that *Alpinia purpurata* plants inoculated with *A. brasilense* presented a greater stem diameter than non-inoculated controls.

On average, sugarcane plants that were inoculated exhibited a better response than non-inoculated plants (Table 4). Hungria *et al*. (2010) also observed variable increases in the productivity of wheat and maize plants that were inoculated with *A. brasilense* (strains Abv5, Abv6 and Abv7, among others) relative to plants that were not inoculated, indicating the positive effect of these bacterial strains in the plant development of these species.

The plants in this study exhibited a better average response to the IC26 inoculant than to the Triazo inoculant for the studied traits (average stalk length, average stalk diameter and Brix). This result was unexpected because associations with diverse bacterial

strains typically promote a greater response from plants, as demonstrated by various studies in the available literature (Oliveira *et al*., 2002; Munir *at al*., 2003; Baldani and Baldani, 2005; Oliveira *et al*., 2009). In the present study, the interaction factor demonstrated that the inoculation response results can vary depending on the family that is examined.

As discussed by Baldani and Baldani (2005) the effect of inoculation on the productivity of plants is dependent on both the plant genotype and the bacterial strain that are used. Thus, different inoculation results are commonly obtained from experiments involving different bacterial strains or sugarcane genotypes. Munos-Rojas and Caballero-Mellado (2003) found diverse results for various productivity traits of sugarcane in an investigation in which they combined various cultivars with seven strains of *G. diazotrophicus*. In particular, these authors found that plants of the variety ME×57-473 that had been inoculated with the PAl3 strain exhibited inferior values of shoot and root dry matter relative to non-inoculated controls. The superior values of these parameters that were observed in the plants that had not been inoculated indicated that the effect of an inoculation can be negative for certain cultivars.

A negative interaction with inoculation was also found in the B4362 sugarcane cultivar. In Brazil, B4362 is the only cultivar that is susceptible to mottled stripe disease, which is caused by the *Herbaspirillum rubrisubalbicans* bacterium (Olivares *et al*., 1997). The inoculation of this cultivar with *Herbaspirillum rubrisubalbicans* results in the cultivar presenting the typical symptoms of mottled stripe disease. This result is consistent with the findings of Urquiaga *et al*. (1992), as these researchers suggested that plant genetic factors can control the bacterial processes of recognition, colonization and nitrogen fixation.

A specific interaction between sugarcane and *A. brasilense* was also observed in a study by Moutia *et al*. (2010), who inoculated two agronomically contrasting cultivars with *A. brasilense* (Azo 195, Azo 249 and Azo 274 strains). These authors observed that the cv. M 1176/77 responded positively to inoculation, whereas, the cv R 570 responded negatively, indicating that the plant genotype needs to be considered in situations involving bacterial inoculation; this result is consistent with the findings of the present study.

The increase of solid soluble compounds (Brix) in plants: The significant differences observed Brix at 14 months after planting indicate that there was generally an increase in Brix in plants that were inoculated with an *A. brasilense* strain, although changes in this characteristic were also significantly impacted by family-bacteria interaction factors (Table 3). More specifically, however, the association of three strains of *A. brasilense* (Triazo) with plants produced decreases in the average Brix of plants, whereas the IC26 inoculation promoted superior values compared with control treatments (T0, not inoculated) (Table 4).

Hari and Srinivasan (2005) observed that the inoculation of certain sugarcane varieties with different species of bacteria (*G. diazotrophicus, Azotobacter* chroococcum and *A. brasilense*) produced higher sugar content in those varieties; however, these authors did not mention the possible factors that may have influenced their results.

In the case of sugarcane, a better understanding of the photosynthetic metabolic processes and the transport and accumulation of metabolites, particularly sucrose, is required (Papini-Terzi *et al*., 2009). It is possible that the higher values of Brix found in plants inoculated with IC26 are related to high nitrogen content, as this strain has constitutive nitrogenase activity; however, it has been found that the split application of N fertilizer at various rates has no significant effect on sugarcane characteristics (Koochekzadeh *et al*., 2009).

The availability of organic N can strongly influence the photosynthesis capacity of plants (Donato *et al*., 2004) and the higher chlorophyll content of sugarcane plants inoculated with *Azospirillum* (Zaied *et al*., 2003; Bashan *et al*., 2006) can be related to their higher photosynthetic rate (Wolff and Floss, 2004). However, additional studies should be conducted to clarify the higher sugar content of plants that have been inoculated with PGPB.

Due to the high number of genotypes evaluated, nitrogen analyses and other meticulous assessment techniques are not viable in the initial phases of the sugarcane breeding program; however, these analytical approaches are recommended for studies of advanced phases of the breeding program to confirm the results that have been obtained.

Family×treatment interactions: The results found in the present work showed significant interaction values between families and inoculation treatments. Certain families presented positive responses to inoculation, such as 4 (RB01616×H64-1881), 37 (RB867515×RB977619), 5 (RB99386×SP91-1049) and 9 (RB845210×RB931003) and some families had negative response 1 (H64-1881×RB92579), 6 (RB001922×RB92579) and 24 (RB966922×polycross).

Moutia *et al*. (2010) also found significant interaction values for the inoculation of two contrasting sugarcane cultivars, R570 and M1176/77, based on a study in which

these cultivars were either treated with an inoculant compound containing three *A. brasilense* strains or left as non-inoculated controls; cultivar performance was then assessed under distinct water regimes (either with or without stress). Although these authors used only one inoculant, the results that they found are consistent with the findings of the present research. In particular, the present study confirms the existence of specific interactions, both positive and negative, between bacteria of the genus Azospirillum and plants of various genotypes.

The interaction observed suggests that sugarcane genotype plays an important role in the success of the PGPB association with plants. Different authors identified genes involved in the recognition of beneficial or pathogenic interactions in sugarcane, such as the SHR5 (Vinagre *et al*., 2006) and scGS1.b genes which were differentially expressed in two contrasting sugarcane cultivars, SP70-1143 (high FBN) and Chunee (low FBN) (Nogueira *et al*., 2005).

The complex genetic structure of sugarcane, which contains duplicated chromosomes and lacks certain chromosomes (poly-aneuploidy), may contribute significantly to the high value of the interactions found in our work, given that the success of the association is sometimes dependent on a single gene. The distinct response of families to the PGPR treatment that is observed in the present work can be linked to the expression of these types of genes.

Other mechanisms are also related to the characteristics of a particular sugarcane cultivar (Baldani and Baldani, 2005), such as the interference of the sugarcane genotype on nitrogenase activity (Ruschel and Ruschel, 1977), the endogenous auxin concentrations and the sensitivity of plant tissues to auxin. In addition, various genotypes can display distinct capabilities for exuding carbon compounds in the rhizosphere (Kennedy, 1999; Hartmann *et al*., 2008), diverse compounds in their root exudates (Moutia *et al*., 2010) and variable capacities to reabsorb these exudates (Benizri *et al*., 2001).

These aforementioned characteristics provide potential mechanisms to explain the variations found in the population of bacteria for each cultivar and the distinct responses of each genotype to inoculation with PGPB.

The significant results found for the interactions with the parameters of Brix and average diameter indicate that in this phase, it is already possible to identify and select families that may be more responsive to inoculation with PGPB. These families which are those that presented high or low interaction values, could be used in future research to discover new mechanisms related to the association between plants and PGPB, as well as possible phenotypic traits or genotypic characteristics that allow for the identification of plants that have particularly positive or negative interactions with PGPB.

The results found in the present work not only put forth the possibility of selecting families and genotypes that are more responsive to bacterial inoculation but also suggest that this selection can be made with the objective of finding the specific ideal interaction between plant genotypes and bacterial strains.

CONCLUSION

The results that were obtained in this work demonstrate that there were significant interactions between the *A. brasilense* strain and the various sugarcane families that were examined. Therefore, in a sugarcane breeding program that seeks to select genotypes with better responses to PGPB inoculation, the inoculation of the seedlings during the first phase of selection is recommended. Moreover, to optimize the interaction between PGPB and plants, the breeder must carefully choose the bacterial strain or strains to use in inoculations of the seedlings in question.

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