The rhizomicrobiome of Sorghum ; impact on plant growth and stress tolerance

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Chapter 5

Effect of *Burkholderia tropica* and *Herbaspirillum frisingense* strains on *Sorghum* growth is plant genotype dependent

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Abstract

Sorghum is a multipurpose crop that is cultivated worldwide. Plant growth-promoting bacteria (PGPB) have important role in enhancing sorghum biomass and nutrient uptake and suppressing plant pathogens. The aim of this research was to test the effects of the endophytic bacterial species *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Pseudomonas fluorescens* strain IAC/BECa 141, *Burkholderia tropica* strain IAC/BECa 135 and *Herbaspirillum frisingense* strain IAC/BECa 152 on the growth and root architecture of four sorghum cultivars (SRN-39, Shanqui-Red, BRS330, BRS509), with different uses and strigolactone profiles. We hypothesized that the different bacterial species would trigger different growth plant responses in different sorghum cultivars. *Burkholderia tropica* and *H. frisingense* significantly increased the plant biomass of cultivars SRN-39 and BRS330. Moreover, cultivar BRS330 inoculated with either strain displayed a significant decrease in average root diameter. This study shows that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

Introduction

Sorghum (*Sorghum bicolor*) is a worldwide-cultivated plant originated in the African continent and later introduced in different parts of the world (Rao *et al.*, 2014). Sorghum has a short growth period, and is therefore a preferred cereal in arid and semi-arid regions (Farre & Faci, 2006, Wu *et al.*, 2010, Funnell-Harris *et al.*, 2013). In Africa, sorghum is mainly cultivated by small farmers as staple food and for beverage production (Haiyambo *et al.*, 2015). By contrast, sorghum is mostly used for the feed market in North and Central America, and for animal feedstock, ethanol production, and soil coverage in South America (Dutra *et al.*, 2013, Perazzo *et al.*, 2013, Damasceno *et al.*, 2014, Rao *et al.*, 2014). Sorghum is currently the 5th most cultivated cereal worldwide (Ramu *et al.*, 2013) and in 2014, approximately 71 million tons of sorghum grains were produced around the world (FAO, 2017).

Sorghum producers often face yield problems due to the soil nutrient deficits, limited access to chemical fertilizers, and the frequent need to combat plant pathogens (Haiyambo *et al.*, 2015). Although conventional agricultural methods, such as chemical fertilization and pesticide application, can be used to overcome these limitations, the environmental side effects of these practices may be unsustainable. As an alternative, the use of plant growth-promoting bacteria (PGPB) as biofertilizers, not only enhances plant biomass and nutrient uptake but also improves pathogen control (Bhattacharyya & Jha, 2012, Dawwam *et al.*, 2013). PGPB can alter the root architecture and promote plant growth by directly facilitating nutrient acquisition or modulating plant hormone levels or by indirectly inhibiting pathogenic organisms (Bhattacharyya & Jha, 2012, Glick, 2012). The best-known processes of plant nutrient acquisition mediated by PGPB are nitrogen fixation, phosphate (P) solubilisation and iron sequestration (Lucy *et al.*, 2004). Different groups of bacteria produce plant growth regulators, such as cytokinins, gibberellins, indole acetic acid (IAA), and ethylene, that may also affect the plant's hormonal balance (Amara *et al.*, 2015). Moreover, PGPB can promote plant growth by fixing N_2 and inhibiting plant pathogens by producing antibiotics or lytic enzymes or competing for resources, which can limit disease incidence and severity (Glick, 2012, da Silveira *et al.*, 2016). In addition to the bacterial modification of plant metabolism, plant exudates have the potential to modify rhizosphere microbial community assembly and interactions (Haichar *et al.*, 2014, Vurukonda *et al.*, 2016). Recent studies suggest that the plant hormone strigolactone (SL) plays an important role in plant rhizosphere bacterial community composition (Funnell-Harris *et al.*, 2008, Schlemper *et al.*, 2017). Furthermore, Peláez-Vico *et al.* (2016) showed that the PGPB *Sinorhizobium meliloti* reduces orobanchol and orobanchyl acetate levels in nodulated alfalfa plants under P

starvation, suggesting a role of SL in rhizobial-legume interactions. Peláez-Vico *et al.* (2016) demonstrated that swarming motility of *S. meliloti* is triggered by the synthetic SL analogue GR24.

Many aspects of the interaction between PGPB and plants have been addressed for a wide range of plant species. Specifically in sorghum, some studies have focused on the interaction of PGPB strains isolated from third-party host species as inoculants for the sorghum rhizosphere (Matiru & Dakora, 2004, Dos Santos *et al.*, 2017), whereas other works have reported the inoculation in other plant species of bacterial strains isolated from sorghum.

Matching beneficial bacteria with their preferred crops might optimize root colonization and biocontrol (Raaijmakers & Weller, 2001), especially when different plants are cropped in soils with the same bacterial composition. In this context, it is extremely important to identify bacterial candidates that have similar growth effects on plants that share the same soil. In Brazil, sorghum have been planted during the sugarcane off season as well as in former sugarcane fields (May *et al.*, 2013), and therefore frequently exposed to the same soil. Endophytic bacteria with plant-growth promoting traits isolated from sugarcane have been shown to increase biomass and plant N content when inoculated in plantlets of sugarcane. Govindarajan et al., 2006 observed an increase in sugarcane yield of 20%, while Sevilla et al. (2001) observed increases of 31% in plant dry matter, 43% in N accumulation, and 25% in productivity in two sugarcane varieties. We hypothesized that different bacterial species isolated from sugarcane will trigger different growth plant responses in different sorghum cultivars. Thus, to determine if endophytic strains characterized as PGPB in sugarcane can act as non-host-specific PGPB benefiting sorghum performance, we tested the effect of five bacterial strains on the plant biomass and root architecture of four *S. bicolor* cultivars with different uses and characteristics: SRN-39, an African grain cultivar that produces high amounts of orobanchol; Shanqui-Red (SQR), a Chinese cultivar that produces high amount of 5-deoxystrigol; BRS330, a hybrid grain cultivar from Brazil and BRS509, a hybrid saccharin cultivar from Brazil that produces both orobanchol and sorgomol (Schlemper *et al*., 2017).

Inoculation of the cultivars SRN-39 and BRS330 with *Burkholderia tropica* or *Herbaspirilum frisingense* strains resulted in significant increases in plant biomass. Moreover, cultivar BRS330 exhibited significant decreases in average root diameter when inoculated with either strain. This study shows that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

Materials and methods

Bacterial isolates and screening of plant growth promotion traits

Five bacterial endophytic strains isolated from sugarcane stems belonging to the Agronomic Institute of Campinas (IAC) – Brazil culture collection were used for this experiment: *Kosakonia radicincitans* strain IAC/BECa-99 (KF542909.1), *Enterobacter asburiae* strain IAC/BECa-128 (JX155407.1), *Pseudomonas fluorescens* strain IAC/BECa-141 (KJ588202.1), *Burkholderia tropica* strain IAC/BECa-135 (KJ670083.1), and *Herbaspirillum frisingense* strain IAC/BECa-152 (JX155400.1).

Phosphate solubilization test: the strains were cultured on a culture medium containing inorganic phosphate ($CaHPO_4$) according to the method of Katznelson & Bose (1959). The experiment was performed in triplicate for five days. The ability of the bacteria to solubilize calcium phosphate was verified by the formation of clear a halo surrounding the colonies.

Indole-3-acetic acid (IAA) test: the strains were grown in culture medium containing L-tryptophan, the precursor of IAA (Bric *et al.*, 1991), covered with a nitrocellulose membrane, and incubated at 28 °C in the dark for 24 h. The nitrocellulose membranes were immersed in Salkowski's solution and incubated at room temperature for up to three hours. This test was performed using five replicates. The formation of a red-purplish halo around the colonies indicated IAA production.

Siderophore production: siderophore production by the strains was measured using the method of Schwyn & Neilands (1987), in which a dye, chromeazurol S (CAS), is released from a dye-iron complex when a ligand sequesters the iron complex. This release causes a colour change from blue to yellow-orange. In this case the ligant was one or more of the siderophores found in the culture supernatants of the bacterial strains. This measurement was made using five replicates.

Hydrogen cyanide (HCN) test: the production of HCN by all strains was assessed according to Bakker & Schippers (1987). Moistened filter paper with picric acid solution (5%) and Na_2CO_3 (2%) was added to the top of the Petri dishes and incubated at 28°C for 36 h. The experiments were performed in triplicate for each strain, and a colour change of the paper from yellow to orange-red indicated the ability to produce HCN.

Sorghum cultivars

Four sorghum cultivars differing in use, origin, and strigolactone production were chosen for inoculation with the selected PGPB. The cultivars were SRN-39, an African sorghum that produces a high amount of orobanchol; Shanqui-Red (SQR) a Chinese sorghum that produces mostly 5-deoxystrigol; BRS330, a hybrid *S. bicolor* grain from Brazil; and BRS509, a hybrid *S. bicolor* saccharin from Brazil that produces both 5-deoxystrigol and sorgomol.

Mesocosm experiment

The experiment was performed in a greenhouse of the Netherlands Institute of Ecology (NIOO-KNAW), located in Wageningen, The Netherlands. The experiment was carried out from September to October 2016, with a total duration of 30 days. A complete random design was used. The treatments consisted of four sorghum cultivars, each one inoculated with each of the five bacterial strains separately, with a total of six replicates per treatment. A non-inoculated treatment under phosphate starvation conditions was established as a control. Seeds were disinfected as described by Liu *et al.* (2013). Briefly, seeds were soaked in 70% ethanol for 3 minutes, transferred to a new tube containing 2.5% sodium hypochlorite solution and shaken for 5 minutes. The seeds were then washed with 70% ethanol solution for 30 s. Finally, the seeds were rinsed with sterile water four times. After the last washing step, 20 µl of the remaining water was plated on Petri dishes with Luria-Bertani (LB) medium to confirm the success of disinfection. After disinfection, the seeds were placed in Petri dishes containing 1% water agar medium, and the plates were incubated at 25 ºC for 2 days in the dark for seed germination. The experiment is illustrated in Figure 1. When radicle emerged from the seed coat, the seedlings were transplanted from the Petri dishes to 11 x 11 x 12 cm plastic pots filled with autoclaved silver sand as substrate. The pots containing one plant each were maintained under greenhouse conditions for four weeks. During the first week, the pots were watered with ½ Hoagland 10% P nutrient solution, followed by P starvation. To create P starvation conditions, the substrate with plants was first flushed with 500 mL of ½-strength Hoagland nutrient solution without phosphate to remove any remaining phosphate in the substrate by drainage through the pot. After two days, to simulate field conditions, 2 g (125 µM) of insoluble tricalcium phosphate ($Ca_3(PO_4)_2$), which can be solubilized by microorganisms but not taken up directly by the plant (Estrada *et al.*, 2013), was diluted in Hoagland nutrient solution and applied to the pots. The watering regime was maintained by applying 25 ml of nutrient solution every two days.

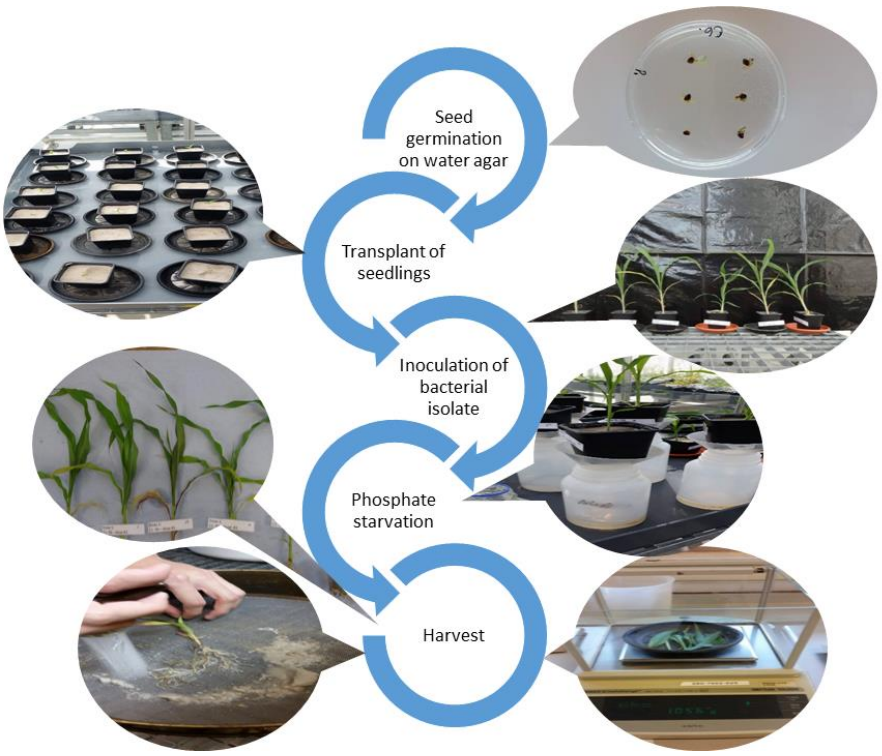

Figure 1. Illustration of the different steps of the study (photos T. Schlemper and F. Silva Gutierrez).

Bacterial inoculation

Bacterial isolates were taken from single colonies, grown in Petri dishes containing Luria-Bertani (LB) medium at 30 ºC for 2-3 days and stored at 4 ºC. Bacterial cells of each strain were then grown overnight at 31 ºC in LB liquid medium and subsequently inoculated again in a fresh LB medium until reaching the desired inoculum density (10^8 cfu ml^{-1}) (Mishra *et al.*, 2016). After transplanting, and during plant growth, bacterial isolates were applied three times on the top of the sandy substrate directly at the location of the seedling roots. The control treatment was inoculation with LB medium without bacteria. The first inoculation was performed on the third day after transplanting, the second on the second day after P starvation, and the last one week later. The inoculation was performed three times to ensure a sufficient bacterial cell density surrounding the plant roots. Loss or dilution of the bacterial inoculum during either the P starvation treatment or the watering regime was possible due to the great drainage potential of the sandy substrate. A density of 10^8 cfu ml^{-1} in a volume of 1ml was used for each bacterial strain at each inoculation time.

Harvesting

Four weeks after transplantation, the experimental plants were harvested, and six plants per treatment were taken for biomass and root architecture measurements. The plants were carefully collected from

the pots and the root system was rinsed with tap water to remove sand particles. The plants were then divided into shoot and root parts for root architecture and plant dry biomass measurements.

Root architecture

For root architecture measurements, the roots were sectioned in three parts, spread along a rectangular acrylic tray and placed in an EPSON scanner Ver. 3.9.3 1NL. The measured root architecture parameters were the specific root area (SRA), specific root length (SRL), average root diameter (AvD), and specific root density (RDENS). All parameters were analysed in WINRHIZO^{TM} program V2005b. Specific root area was calculated by dividing the surface area by the root dry biomass. Specific root length was calculated by the following formula:

$$SRL = \frac{\frac{\text{root length}}{100}}{\text{root dry biomass}} \times 10$$

Plant biomass

The shoot and root parts were dried at room temperature for four hours, until no remaining water could be observed on their surfaces. The fresh weights of both parts were obtained using an electronic scale. The shoot and root parts were then placed in an oven at 60 ºC for 72 hours. The percentage of biomass was calculated by dividing the dry weight biomass by the fresh weight and multiplying by 100.

Statistical analysis

The plant dry biomass and root architecture data were analysed by analysis of variance (ANOVA) and Duncan's test ($P<0.05$) using the IBM SPSS Statistics 23 program.

Results

Bacterial strains and PGPB effect on sorghum plant biomass

The five bacterial strains exhibited different characteristics in terms of specific plant growth-promotion traits (Table1). Strain IAC/BECa 128 (*Enterobacter asburiae*) had the capability to solubilize phosphate. All strains produced IAA, except strain IAC/BECa 135 (*Burkholderia tropica*). Strains IAC/BECa 99 (*K. radicincitans*), IAC/BECa 128 (*E. asburiae*) and IAC/BECa 152 (*H. frisingense*) produced siderophores. Strain IAC/BECa 141 (*Pseudomonas fluorescens*) produced hydrogen cyanid.

Table 1. Plant growth promotion characteristics of five bacterial isolates IAC/BECa 99 (*Kosakonia radicincitans*), IAC/BECa 128 (*Enterobacter asburiae*), IAC/BECa 135 (*Burkholderia tropica*), IAC/BECa 141 (*Pseudomonas fluorescens*) and IAC/BECa 152 (*Herbaspirillum frisingense*)

PGPB trait	Strain				
	IAC BECa 99	**IAC BECa 128**	**IAC BECa 135**	**IAC BECa 141**	**IAC BECa 152**
P Solubilization	-	+	-	-	-
IAA	+	+	-	+	+
Siderophore	+	+	-	-	+
Hydrogen cyanid	-	-	-	+	-
nifH gene	+	-	-	-	-

Positive (+) and negative (-) signals mean positive and negative results for each plant growth promotion trait listed

Sorghum cultivar SRN39 exhibited a significant increase in root dry biomass when inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152, and a significantly higher shoot biomass when inoculated with *E. asburiae* strain IAC/BECa 128 or *H. frisingense* strain IAC/BECa 152, compared with the control (Table 2). Cultivar BRS330 displayed a significant increase in root dry biomass when inoculated with strain IAC/BECa 135 (*B. tropica*) or IAC/BECa 152 (*H. frisingense*) compared with the control. However, when inoculated with *E. asburiae* strain IAC/BECa 128, exhibited a significant decrease in shoot biomass compared with the non-inoculated control. Cultivars SQR and BRS509 did not exhibit significant differences in biomass when inoculated with any of the strains compared to the control (Table 2).

Table 2: Root and shoot biomass (%) of four cultivars of sorghum (SRN-39, SQR, BRS330 and BRS509) inoculated with five bacteria *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Burkholderia tropica* strain IAC/BECa 135, *Pseudomonas fluorescens* strain IAC/BECa 141 and *Herbaspirillum frisingense* strain IAC/BECa 152.

Cultivars	**Bacterial Isolate**	**Root Biomass (%)**	**Shoot Biomass (%)**
SRN-39	CONTROL	18.10 ± 1.19 c	22.20 ± 0.73 c
	IAC/BECa 99	21.52 ± 0.75 bc	23.66 ± 0.50 bc
	IAC/BECa 128	24.83 ± 3.03 abc	25.22 ± 0.98 b
	IAC/BECa 135	27.48 ± 3.20 ab	23.82 ± 0.37 bc
	IAC/BECa 141	21.46 ± 2.06 bc	23.94 ± 0.68 bc
	IAC/BECa 152	31.47 ± 1.74 a	28.51 ± 1.34 a
SQR	CONTROL	29.52 ± 2.84 a	22.21 ± 0.88 a
	IAC/BECa 99	24.50 ± 2.25 a	19.62 ± 1.15 a
	IAC/BECa 128	25.67 ± 1.90 a	19.56 ± 0.97 a
	IAC/BECa 135	31.15 ± 3.24 a	19.69 ± 0.64 a
	IAC/BECa 141	29.26 ± 3.56 a	20.31 ± 0.87 a
	IAC/BECa 152	33.64 ± 1.59 a	20.94 ± 0.37 a
BRS330	CONTROL	13.19 ± 0.69 bc	20.75 ± 0.35 a
	IAC/BECa 99	12.58 ± 0.48 bc	19.92 ± 0.54 ab
	IAC/BECa 128	11.77 ± 0.69 c	19.82 ± 0.32 b
	IAC/BECa 135	19.17 ± 2.30 a	21.24 ± 0.33 a
	IAC/BECa 141	16.14 ± 1.02 ab	19.91 ± 0.48 ab
	IAC/BECa 152	18.43 ± 0.98 a	20.50 ± 0.30 ab
BRS509	CONTROL	24.13 ± 2.00 ab	25.38 ± 1.46 a
	IAC/BECa 99	20.79 ± 2.60 b	23.56 ± 0.23 a
	IAC/BECa 128	24.57 ± 0.88 ab	22.73 ± 0.65 a
	IAC/BECa 135	28.29 ± 2.38 a	22.48 ± 0.44 a
	IAC/BECa 141	20.68 ± 1.62 b	22.97 ± 0.48 a
	IAC/BECa 152	24.04 ± 1.70 ab	23.44 ± 1.50 a

The values are means of replicates (n=6) ± (SE). For each parameter, letters compare (on column) the means between the bacterial inoculums treatments within the same cultivar. Means followed by the same letter are not statistically different by Duncan test ($P<0.05$).

PGPB effect on root architecture

Cultivars SRN39, SQR, and BRS509 did not display significant differences in root architecture parameters when inoculated with any strain compared with the control. However, cultivar BRS330 inoculated with *E. asburiae* strain IAC/BECa 128 exhibited a significantly higher specific root area (SRA) and specific root length (SRL) compared with the control. Furthermore, when inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152, the same cultivar exhibited a significant decrease in root average diameter (AvD) compared with the control (Table 3). Cultivar BRS 330 inoculated with *B. tropica* (IAC/BECa 135), *P. fluorescens* (IAC/BECa 141) or *H.*

frisingense (IAC/BECa 152) had a higher specific root density (SRD) compared than the treatment inoculated with *E. asburiae* (IAC/BECa 128), but not the control.

Table 3: Specific root area (SRA), specific root lenght (SRL), average of root diameter (AvD) and specific root density (RDENS) four cultivars of sorghum (SRN-39, SQR, BRS330 and BRS509) inoculated with five bacteria *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Burkholderia tropica* strain IAC/BECa 135, *Pseudomonas fluorescens* strain IAC/BECa 141 and *Herbaspirillum frisingense* strain IAC/BECa 152.

Cultivars	**Isolates**	**SRA (cm2/g)**	**SRL (cm/g)**	**AvD (mm)**	**RDENS (cm3/g)**
SRN-39	CONTROL	847.29 ± 44.46 a	687.39 ± 66.85 a	0.40 ± 0.02 a	0.12 ± 0.00 a
	IAC/BECa 99	1027.44 ± 130.77 a	867.21 ± 151.8 a	0.39 ± 0.02 a	0.11 ± 0.01 a
	IAC/BECa 128	1061.95 ± 146.42 a	881.21 ± 66.73 a	0.38 ± 0.03 a	0.11 ± 0.02 a
	IAC/BECa 135	1016.59 ± 59.11 a	882.89 ± 91.54 a	0.38 ± 0.02 a	0.11 ± 0.01 a
	IAC/BECa 141	1044.78 ± 68.45 a	883.43 ± 67.29 a	0.38 ± 0.02 a	0.10 ± 0.01 a
	IAC/BECa 152	914.74 ± 50.78 a	802.62 ± 69.77 a	0.37 ± 0.01 a	0.12 ± 0.00 a
SQR	CONTROL	1021.64 ± 41.81 a	909.61 ± 69.28 a	0.36 ± 0.01 a	0.11 ± 0.00ab
	IAC/BECa 99	1034.82 ± 58.91 a	922.61 ± 62.72 a	0.36 ± 0.01 a	0.11 ± 0.01ab
	IAC/BECa 128	1217.28 ± 204.39 a	1220.4 ± 310.4 a	0.35 ± 0.02 a	0.10 ± 0.01ab
	IAC/BECa 135	1239.66 ± 109.67 a	1056.5 ±116.53 a	0.38 ± 0.02 a	0.09 ± 0.01 b
	IAC/BECa 141	1284.28 ± 207.40 a	1214.2 ± 211.88a	0.34 ± 0.01 a	0.10 ± 0.01 b
	IAC/BECa 152	917.84 ± 26.31 a	894.24 ± 32.09 a	0.33 ± 0.01 a	0.13 ± 0.00 a
BRS330	CONTROL	881.00 ± 28.55 b	609.24 ± 23.55 b	0.46 ± 0.01 a	0.10 ± 0.00ab
	IAC/BECa 99	926.69 ± 66.26 ab	677.58 ± 47.12ab	0.43 ± 0.01ab	0.10 ± 0.01ab
	IAC/BECa 128	1101.94 ± 96.50 a	774.88 ± 72.32 a	0.46 ± 0.01 a	0.08 ± 0.01 b
	IAC/BECa 135	841.07 ± 80.40 b	645.94 ± 60.04ab	0.41 ± 0.01 b	0.12 ± 0.01 a
	IAC/BECa 141	767.82 ± 44.72 b	561.31 ± 32.04 b	0.44 ± 0.01ab	0.12 ± 0.01 a
	IAC/BECa 152	786.94 ± 14.98 b	605.28 ± 18.08 b	0.42 ± 0.01 b	0.12 ± 0.00 a
BRS509	CONTROL	1337.8 ± 665.09 a	1121.9 ± 531.2 a	0.38 ± 0.03ab	0.17 ± 0.04 a
	IAC/BECa 99	707.81 ± 68.59 a	553.35 ± 62.87 a	0.41 ± 0.01 a	0.14 ± 0.01 a
	IAC/BECa 128	633.89 ± 45.40 a	524.96 ± 35.46 a	0.38 ± 0.01ab	0.17 ± 0.01 a
	IAC/BECa 135	779.74 ± 53.88 a	662.51 ± 50.39 a	0.38 ± 0.01ab	0.14 ± 0.01 a
	IAC/BECa 141	1130.4 ± 230.66 a	1138 ± 325.22 a	0.34 ± 0.02 b	0.12 ± 0.01 a
	IAC/BECa 152	703.37 ± 79.40 a	622.32 ± 74.82 a	0.36 ± 0.01ab	0.17 ± 0.02 a

Values are means of replicates (n=6) ± (SE). For each parameter, letters compare (on column) the means between the bacterial inoculum treatments within the same cultivar. Means followed by the same letter are not statistically different by Duncan test ($P<0.05$).

Discussion

This work aimed to evaluate the effect of five bacterial strains isolated from sugarcane on the plant growth and root architecture of four sorghum cultivars. Cultivars SRN-39 and BRS330 inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152 and cultivar SRN-39 inoculated with *E. asburiae* strain IAC/BECa 128 or *H. frisingense* strain IAC/BECa 152 exhibited

significant increases in sorghum root and shoot biomass, respectively, compared with the control. Although the number of replicates in our study was small (6) our results corroborate those of Chiarini *et al.* (1998), who found that isolates belonging to the genera *Burkholderia* and *Enterobacter* co-inoculated in the sorghum rhizosphere promoted a significant increase in root growth compared to non-inoculated plants. Furthermore, species belonging to the genera *Burkholderia* and *Herbaspirillum* promote the growth of sugarcane and maize (Pereira *et al.*, 2014, da Silva *et al.*, 2016), which like sorghum, are C4 grass species. *Herbaspirilum frisingense* strain IAC/BECa 152 produces siderophores and IAA, whereas *B. tropica* strain IAC/BECa 135 does not. The strain IAC/BECa 152 might possess a set of mechanisms that improve plant nutrient uptake either by increasing nutrient availability in the rhizosphere or influencing the biochemical mechanisms underlying nutritional processes (Pii *et al.*, 2015). Such mechanisms include changes in the root system architecture and shoot-to-root biomass ratio, increases in proton efflux by modulating H+APTase activities, indirect effects of IAA produced by PGPB, or acidification of the rhizosphere to enhance nutrient solubility (Pii *et al.*, 2015). In addition to growth regulators, siderophores can be produced and are known to assist Fe acquisition by roots (Saravanan *et al.*, 2007, Mehnaz *et al.*, 2013).

In contrast to the effects of *B. tropica*, *H. frisingense* and *E. asburiae* on the growth of both grain sorghum cultivars, these strains had no significant effect on BRS 509 (sweet sorghum) and on SQR cultivars compared with the control. Interestingly, in accordance with our results, Dos Santos *et al.* (2017) observed significant increase in the biomass of grass and grain sorghum inoculated with *Burkholderia* ssp. or *Herbaspirillum* ssp. but not sweet sorghum inoculated with the same isolates. Taken together with our results, these findings suggest that the effects of strains of *B. tropica* and *H. frisingense* on plant growth are dependent on sorghum genotype. It is unclear why the effects of these strains were greater in certain sorghum cultivars than others. However, different sorghum genotypes release different strigolactone molecules in different quantities under P starvation (Schlemper *et al.*, 2017). Thus, the high relative abundance of the genus *Bulkholderia* in the rhizosphere of sorghum cultivar SRN-39 could be related to the level of orobanchol, which is 300 and 1100 times higher in SRN-39 than in the cultivars SQR, BRS330 and BRS509 as suggested by Schlemper *et al.* (2017).

When inoculated with *E. asburiae* strain IAC/BECa 128, cultivar BRS330 displayed an increase in SRL and area compared with the control. These finding are in agreement with a study by Kryuchkova *et al.* (2014), who reported that *Enterobacter* species can promote increases root length and lateral roots in sunflower. The strain IAC/BECa 128 can solubilize phosphate, which trait might explain the increase in root biomass. The cultivar BRS330 inoculated with strain IAC/BECa 135 (*B.*

tropica) or IAC/BECa 152 (*H. frisingense*) exhibited a significant decrease in average root diameter compared with the control but slight increase in root density. Plants under P deficiency conditions may increase root density probably to enhance nutrient acquisition (Kapulnik & Koltai, 2014).

Moreover, species belonging to the genus *Herbaspirillum* can influence plant root architecture and improve signalling pathways of plant hormone production (Straub *et al.*, 2013). No significant effects on sorghum growth or root architecture modification were observed when *K. radicincitans* strain IAC/BECa-99 or *P. fluorescens* strain IAC/BECa-141 was inoculated in the rhizosphere of any evaluated sorghum cultivar. Although there are many reports on the effects of *K. radicincitans* (formerly known as *Enterobacter radicincitans*) on a range of plants, such as *Arabidopsis thaliana*, radish, and tomato (Berger *et al.*, 2013, Brock *et al.*, 2013, Berger *et al.*, 2015), there are no reports on the effects of this bacterial species on sorghum growth or root architecture modification. With respect to *P. fluorescens*, Marcos *et al.* (2016) found that the strain IAC/BECa 141, when used as an inoculant applied to two sugarcane varieties, increased chlorophyll *a* content without changing plant growth. Kumar *et al.* (2012) studying the effect of seven different fluorescent *Pseudomonas spp.* strains with single or multiple PGPR traits, in sorghum growth, observed that all strains were able to increase sorghum growth compared to a non-inoculated control.

Our results demonstrated that selected bacterial strains characterized as PGPB in sugarcane were able to promote plant growth and root architecture modification in sorghum. Based on the reproducibility of the performance of bacterial strains for different crops, our findings shed light on the identification of bacterial candidate strains for improving the growth and yield of crops that share the same soil bacterial source in intercropping or crop rotation systems. However, since we did not evaluate the bacterial community that actually colonized the root system, we strongly recommend future studies to recover the bacterial community from the endosphere and rhizosphere compartments as proof of the effectiveness of the inoculation. We suggest that SL plays a role in the effectiveness of PGPB in promoting the growth of specifics sorghum genotypes, although our experimental set-up did not allow us to make a straight forward conclusion. More specific experiments are needed to better address the relationship between plant strigolactone production and plant bacterial infection.

Conclusion

Here we demonstrated that bacteria strains characterized as PGPB in sugarcane were able to promote plant growth and root architecture modification in sorghum. Our results demonstrated that cultivars SRN-39 and BRS330 inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain

IAC/BECa 152 exhibited a significant increase in plant biomass. Moreover, cultivar BRS330 inoculated with either strain displayed a significant decrease in AvD. The results of this study indicate that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

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