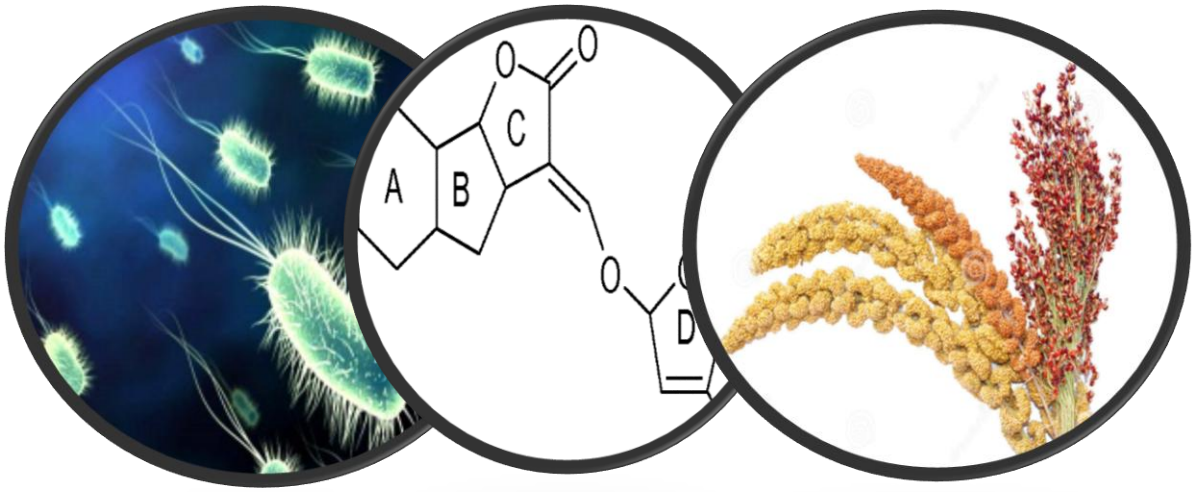
# PLANT GROWTH PROMOTING BACTERIA AND STRIGOLACTONE INTERACTIONS

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## Abstract

Sorghum is a crop that has been cultivated for multiple purpose worldwide. However, biotic problems during cultivation, such as parasitic plant infestation, have been threaten their production. Resistance mechanisms against parasitic plants have been related with production and type of strigolactone (SL) by different sorghum cultivars. Despite SL influence arbuscular mycorrhizal fungi (AMF) hyphal branching, there is few available data of SL influencing beneficial bacteria movement. In this way, the aim of this research was to select beneficial bacteria for sorghum cultivars with different SL profile and study the bacterial motility under the presence of SL synthetic analogue GR24. For this purpose, it was set a bioassay to test plant growth promotion by bacterial isolates from *Burkolderia, Kosakonia, Herbaspirillum, Enterobacter* and *Pseudomonas* genus in four sorghum cultivars with different profiles of orobanchol and 5-deoxystrigol. Moreover, an in-vitro assay was performed testing the selected bacterial isolates under different concentrations of GR24. Results showed that *B. tropica* and *H. fringiensis* have plant growth promotion influence in two out of four sorghum cultivars. Moreover, in vitro experiment showed that *B. tropica* and *K. radicincitans* motility were influenced by GR24. Findings in this research can be the first steps for further investigations related to SL- beneficial bacteria interaction.

## Introduction

Sorghum (*Sorghum bicolor*) is a worldwide-cultivated plant originated in the African continent (De Wet & Harlan, 1971). In Africa, people use sorghum mainly as staple food, but in other parts of the world like Brazil, it can be used as animal feed (Henley, 2010). The domesticated sorghum has been introduced in different parts of the world, including Europe (Musisi, 2011).It has been estimated that in 2016, sorghum production will be 67.61 million metric tons, therefore, sorghum is considered one of the most producer grains in the world (FAOSTAT, 2016) . However, parasitic plants, such as *Striga hermonthica* (striga) and *Orobanche minor*, have been threaten the potential yield of sorghum plant. In this way, researchers have been working on some strategies to cope this problem (Yoneyama, et al., 2010).

Different sorghum varieties that can be found in the nature developed different strategies for adaptation to biotic and abiotic threatens. Therefore, it has been recommended to make developmental breeding program using striga resistance varieties to fight against parasitic plant problems (Mohemed et al., 2016). For instance, Mohamed, Housley, and Ejeta (2010) reported some varieties, like CK32 and KP33 have a strong hypersensitive response against striga infections, being potential candidates for the breeding program. Another example is mentioned by Ezeaku and Gupta (2004), wheresorghum variety SRN39 has developed resistance to parasitic plant *Striga hermonthica*, compared to the wild-type. Interestingly, the interaction of striga and abovementioned sorghum cultivars are related with the production of strigolactones (SL) (Alder et al., 2011;Mohemed et al., 2016).

SL are carotenoid, derived compounds that are considered plant hormones (Delaux et al., 2012). These molecules are exuded by plant roots when plants face nutrient deficient conditions, especially phosphate limitation (H. Koltai, 2013) . Furthermore, SLs can lead to morphological changes in the plant, such regulation of the stems branching, root elongation, root hair formation, increase stem thickness and boost leaf senescence (Al-Babili & Bouwmeester, 2015; Brewer et al,, 2013; Jamil et al., 2012; Hinanit Koltai, 2011). Different types of SL, like strigol, strigyl acetate, orobanchol, sorgomol, and solanacol have been detected in exudates of sorghum, maize, cotton and millet plants (Akiyama et al., 2010; Yoneyama et al., 2010).

Moreover, in-vitro assays showed that germination stimulant of striga seed (Fig. 1) might differ, depending on SL molecules. Therefore, it is assumed that there is a correlation between different SLs and their ability to induce different levels of striga susceptibility sorghum plants (Yoneyama et al., 2010). (Mohemed et al., 2016; Vogler et al., 1996; Yoneyama et al., 2010). For instance, high orobanchol producing lines like SRN39 are more resistant to *Striga* comparing to six other cultivars with low orobanchol production (Mohemed et al., 2016). In this way, there are relevant information testing that SL types and amounts are related with plan-parasite interaction.

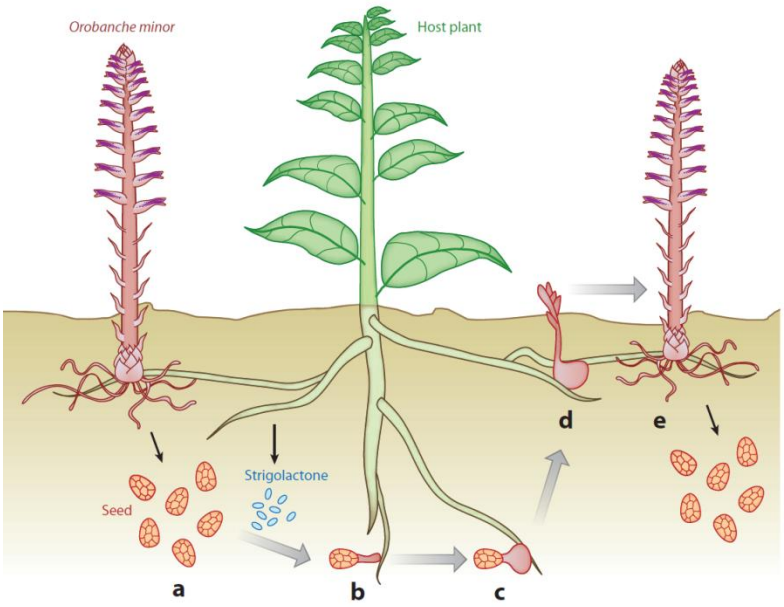


Fig 1. : *Orobanche minor* parasitic cycle (Yoneyama et al., 2010)

Despite the role of SL in plant morphology modification and stimulation striga seed germination (Fig. 1) ,SL was described as promotor of AMF hyphal branching (Yoneyama et al., 2010). Studies suggested that under phosphate (P) starvation, plants start producing SL in order to recruit AMF to facilitate the uptake of insoluble P that the host cannot easily achieve (Dawwam et al., 2013) (Fig 2). However, as it was mentioned before, interaction of SLs with parasitic plants or AMF can vary depending on the structure of SL molecule (Akiyama et al., 2010).

Moreover, recent studies relates the bacterial movement (motility) with the presence of SL molecules. For instance, the presence of *S. meliloti* reduce the orobanchol and orobanchyl acetate levels in alfalfa nodulated plants under P starvation, suggesting that SL might play

certain role in rhizobial-legume interaction. Moreover, in vitro assays showed that swarming motility of *Sinorhizobium meliloti* is triggered by synthetic SL analogue GR24 in concentration dependent manner (Pelaez-Vico et al., 2016).

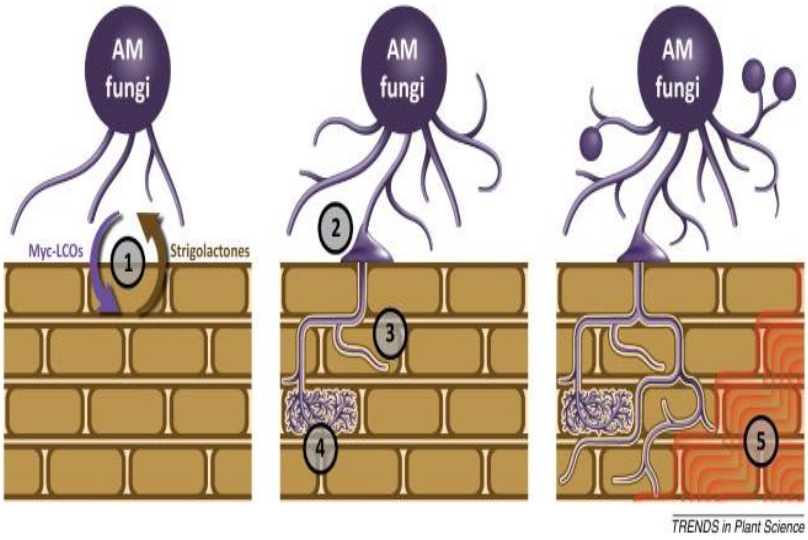


Fig. 2 : Arbuscular Mycorrhizal Fungi - plant cycle (Delaux et al., 2013).

Bacteria motility can be one of the important traits when host colonization, attachment and migration take place, and can be categorize as swimming and surface motility (Czaban et al., 2007; Turnbull et al, 2001). Swimming motility is the individual bacterial basic movement in aquatic environment (Venieraki et al., 2016), and is usually tested in vitro using low concentration agar media (Pelaez-Vico et al., 2016). On the other hand, surface motility is a multicellular movement of the flagellated bacteria complexes on solid surfaces (Venieraki et al., 2016). Overall, motility is an advantageous bacteria characteristic to colonize roots and rhizosphere, on water saturated and not saturated soils (Venieraki et al., 2016). Furthermore bacterial movements were not only traits of pathogenic bacteria but also other types of bacteria that contribute beneficial effects to the plant (Venieraki et al., 2016; Pelaez-Vico et al., 2016).

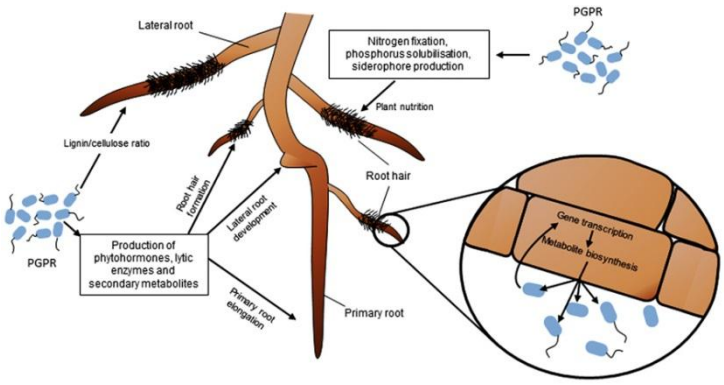


Fig. 3 : Plant growth promoting bacteria - plant cycle (Vacheron et al., 2013)

Some bacterial species, like *S. meliloti*, are called plant growth promoting bacteria (PGPB) because they contribute in different ways to improve the biomass of plants (Fig 3) (Dawwam et al., 2013) (Liu et al., 2013) . For instance, *Rhizobium* is one of the bacterial genus that fix nitrogen making it available for the plant. Regarding P uptake, Malfanova (2013) mentioned that *Bacillus megaterium*, *Pseudomonas* spp, and *Pseudomonas fluorescens* B16 solubilize the P that is not available for the crops. Some other pseudomonas species chelate iron using siderophores, and make them available for the plant (Malfanova, 2013). Other studies proved that *Bacillus cereus* and *Achromobacter xylosoxidans*, not only solubilize phosphorus, also were able to produce hormone precursor Idol-3-acetic acid (IAA) and increase the efficiency of plant growth promotion. (Dawwam et al., 2013). In short, many species are classified as PGPB, because they help improvement, by different mechanisms, in the plant productivity adaptation to different environmental conditions (Akiyama et al., 2010; Dawwam et al., 2013).

The aim of this project was to relate selectivity of beneficial bacterial isolates for different sorghum cultivars based on SL profile. In this way, it was expected that beneficial bacteria would promote plant growth, in terms of plant biomass and root architecture, in specific sorghum cultivars. Furthermore, the influence of SL synthetic analogue GR24 in bacterial isolates movement was also evaluated in terms of swimming and surface motility. Thus, it would show that SL would trigger or repress bacterial movements in laboratory conditions.

## Materials and methods

### Biological material to use: sorghum cultivars and bacterial isolates.

I used five bacterial isolates during the experiments. Bacterial isolates were isolated from stem of sugarcane plants. The name of isolates were 99 (*Kosakonia radicincitans*), 128 (*Enterobacter asburiae*), 135 (*Burkholderia tropica*), 141 (*Pseudomonas fluorescens*), and 152 (*Herbaspirillum frisingense*). Isolates selection showed plant growth promotion in inoculated sugarcane plants. For working purpose, I grew bacterial isolates in petri dishes containing LB media, incubated for 2-3 days at 30 ºC and then stored at 4 ºC.

I chose four sorghum cultivars in a plant growth promotion (PGP) experiment (greenhouse), based on strigolactone production profile and commercial use. The sorghum cultivars were the African SRN-39 that produce high amounts of orobanchol, and the Chinese SQR to produce 5-deoxystrigol. Moreover, I selected Brazilian sorghum cultivars such as, a hybrid grain of *S. bicolor* BRS330 (C5), and a hybrid saccharin of *S. bicolor* BRS509 (C6) based on their commercial use.

### Bioassay Experimental site

I performed the PGP experiment in a greenhouse of the Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands. I carried out the experiment from September to October 2016 using complete random design. Treatments consisted of 4 sorghum cultivars, each one inoculated with 5 bacterial isolates, having in total 9 replicates per treatment. For each treatment, I establish a control under phosphate starvation condition, and a positive control under complete nutrient application.

Disinfection of seeds was performed as previously described by Liu et al. (2013). Briefly, seeds were soaked in ethanol 70% for 3 minutes, transferred to a new tube containing sodium hypochlorite solution 2.5% and the tube was shaken for 5 minutes. After that, I washed the seeds in a new ethanol 70% solution for 30 seconds. Finally, I rinsed them with autoclaved

water four times. After the last washing step, 20 uL of the remaining water was plated on petri dishes in order to check the success of the disinfection.
After the disinfection, seeds were placed in petri dishes containing 1% of water agar medium, and plates were kept at 25 ºC for 2 days in the dark. When radicles were out of the seeds coat, seeds were transplanted from petri dishes to plastic pots.

Seedling were transplant to 11x11x12 square cm plastic pots filled with autoclaved silver sand as substrate. The pots were maintained in the under greenhouse conditions for 3 weeks under ½ Hoagland 10% P nutrient solution application.

After 14 days, I applied bacteria isolates to the soil. Regarding bacterial preparation, bacterial cultures grew overnight at 31ºC in Luria-Bertani (LB) medium, then inoculated again in a fresh LB medium and grew until reaching a value of 0.6 (OD600) with an inoculum density of $10^8$ cfu.ml$^{-1}$ (Mishra et al., 2016). Bacterial isolates were applied three times during the whole period of the experiment. The first bacterial inoculation was the third day after transplant. The second bacterial inoculation was carried out at second day after starting P starvation, and the last bacterial inoculation, one week later.

P starvation treatment started one week after the first bacteria application to the plants. Firstly, the substrate with plants was flushed using 500 mL of ½-strength Hoagland nutrient solution without phosphate. In this way, possible phosphate that could remain on substrate could be drained through the pot. After two days, in order to simulate the field conditions, where immobilized phosphate can be solubilized by microorganisms and used by plants, 30 mL of insoluble phosphate (Ca3(PO4)2) was applied to the pots.

## Harvesting

After 4 weeks of transplant, the experiment was harvested and six plants per treatment were taken for biomass and root architecture measurements. Plants were extracted from the pots carefully and had their root system rinsed with tap water. Then plants were dried at room temperature, until no remains of water could be seen on their surface. For biomass measurements, plants were split in root and shoot parts. Both parts were fresh weighted using an electronic scale. After that, they were stored in an oven at 60 ºC for 3 days. Percentage of

biomass was the first parameter to evaluate in this experiment. Regarding calculation of biomass, dry weight divided by the fresh weight and then multiplied by 100. Both root and shoot biomass were measured.

For root architecture measurements, I sectioned the roots system in three parts and spread along a rectangular acrylic tray and placed in an EPSON scanner Ver. 3.9.3 1NL. Some parameters to measure were root diameter, root specific area, surface area, root specific length, and root density. All of these parameters were analysed in WINRHIZO$^{TM}$ program V2005b. Root specific area calculation was: surface area dividing by the root dry biomass. Root specific length calculation was: root length dividing by 100 and then by root dry biomass, all together multiplied by 10.

## Influence (in vitro) of GR24 on bacterial isolates

This experiment of swimming and surface motility was based on the adjusted protocol from Pelaez-Vico et al. (2016). Three different concentration of SL compound were tested for each bacterial isolate. Two control treatments were established: one consisted in apply water rather than SL compound, and the other consisted of acetone in the same amount used to dilute SL compounds. Each treatment had 3 biological replicates.

Bacto media (BM) containing 0.3% bacto agar was supplemented with three different concentration of synthetic SL compound GR24 (1, 0.1, and 0.01 uM final concentration) and then plated in petri dishes. Acetone with the same final concentrations and demi water were considered for control treatments. Bacterial growth procedure was adjusted from Pelaez-Vico et al. (2016). Bacterial cultures grew overnight in Luria-Bertani (LB) medium at 31ºC, from this, it was inoculated again in a fresh liquid medium and grew until OD 0.6 (OD600) . After that, 20 uL of inoculum was applied in the middle of the petri dish containing the prepared media. The bacterial motility evaluation (colony diameter in mm) was done after 2 days of inoculation.

In surface motility experiment, it was performed on semi solid plates of minimal media (MM) containing 0.6% agar and supplemented with synthetic compound GR24 (1, 0.1, and 00.1 uM

final concentration). The control consisted of diluted acetone and water. Bacterial growth was followed as it was mentioned in previous paragraph. However, after reaching OD 0.6, they were pelleted, washed and re-suspended in 0.1 volume of saline solution. Aliquots of 2uL from the bacteria inoculum were placed in the middle of opened petri dish letting it dry for some minutes. After the excess water has evaporated, the plates were incubated for 2 days at 31C, and colony diameter (mm) was measured.

### Statistical analysis

Data of biomass collection, root architecture of the bioassay, and bacterial diameter for in vitro experiment were analysed using Duncan test from IBM SPSS Statistics 23 program.

## Results

### PGPR effect on different cultivars of sorghum:

In cultivar C1, the inoculation of the isolate 152 (*H. frisingense*) and 135 (*B. tropica*) caused a significant increase in root biomass (Table 1), compared with the control. However, in cultivar C2, no significant differences could be found when plant were inoculated with any of the isolates, comparing with the control. In cultivar C5, there was a significant increase in the root biomass for inoculations with isolates 135 (*B. tropica*) and 152 (*H. frisingense*), comparing with the control. Even though, the inoculation of isolate 135 (*B. tropica*) significantly increase root biomass in cultivar C6, there is no difference with plants from control.

Regarding percentage shoot biomass (%) (Table 1) in C1 plants, the inoculation with isolate 135 (*B. tropica*) and 128 (*E. asburiae*) were significantly higher than control treatment. In C2 plants, there were no significant difference with any of the inoculations comparing with the control. In C5 plants, inoculation of isolates 152 (*H. frisingense*) and 128 (*E. asburiae*) had significant higher values comparing with the control plants. In C5 plants, inoculation with isolate 128 (*E. asburiae*) lead to a significant decrease in the shoot biomass comparing to the

control. Moreover, in C6 plants, there was no significant difference from inoculated plants of any treatment comparing to the control.

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Table 1: **Biomass (%) of four cultivars of sorghum inoculated with five bacterial isolates**

| Cultivars | **Bacterial Isolate** | **Root Biomass (%)** | **Shoot Biomass (%)** |
|---|---|---|---|
| C1 | CONTROL | 18.10 ± 1.19 c[1] | 22.20 ±0.73 c |
| | 99 | 21.52 ± 0.75 bc | 23.66 ± 0.50 bc |
| | 128 | 24.83 ± 3.03 abc | 25.22 ±0.98 b |
| | 135 | 27.48 ± 3.20 ab | 23.82 ± 0.37 bc |
| | 141 | 21.46 ± 2.06 bc | 23.94 ±0.68 bc |
| | 152 | 31.47 ± 1.74 a | 28.51 ±1.34 a |
| C2 | CONTROL | 29.52 ± 2.84 a | 22.21 ±0.88 a |
| | 99 | 24.50 ± 2.25 a | 19.62 ± 1.15 a |
| | 128 | 25.67 ± 1.90 a | 19.56 ±0.97 a |
| | 135 | 31.15 ± 3.24 a | 19.69 ±0.64 a |
| | 141 | 29.26± 3.56 a | 20.31 ±0.87 a |
| | 152 | 33.64 ± 1.59 a | 20.94 ± 0.37 a |
| C5 | CONTROL | 13.19 ± 0.69 bc | 20.75 ±0.35 a |
| | 99 | 12.58 ± 0.48 bc | 19.92 ±0.54 ab |
| | 128 | 11.77 ± 0.69 c | 19.82 ±0.32 b |
| | 135 | 19.17 ± 2.30 a | 21.24 ± 0.33 a |
| | 141 | 16.14 ± 1.02 ab | 19.91 ± 0.48 ab |
| | 152 | 18.43 ± 0.98 a | 20.50 ±0.30 ab |
| C6 | CONTROL | 24.13 ± 2.00 ab | 25.38 ±1.46 a |
| | 99 | 20.79 ± 2.60 b | 23.56 ± 0.23 a |
| | 128 | 24.57 ± 0.88 ab | 22.73 ±0.65 a |
| | 135 | 28.29 ± 2.38 a | 22.48 ± 0.44 a |
| | 141 | 20.68 ± 1.62 b | 22.97 ±0.48 a |
| | 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 ($P<0.05$).

In specific root area (SRA) and specific root length (SRL) (Table 2), the inoculations did not cause any significant change in cultivars C1, C2, and C6. However, for C5 plants, there is a significant increase in SRA and SRL in inoculated plants with isolate 128 (*E. asburiae*), comparing with control.

Regarding root average diameter (Table 2), none of the inoculation cause significant differences comparing to the treatment in C1 and C2 plants. In C5, inoculations with isolates 135 (*B. tropica*) and 152 (*H. frisingense*) cause a significant reduce in root average diameter significant, comparing to the control. Furthermore, in C6 plants, only inoculation of isolate 141 (*P. fluorescens*) cause a reduction in the root average diameter comparing with the control.

While analysing Specific Root Density (SRD) (Table 2), none of five bacterial isolates cause any significant change comparing with the control, in cultivars C1, C2, C5 and C6.

Table 2**: Root architecture traits of four cultivars of sorghum inoculated with five bacterial isolates**

| Cultivars | Bacterial Isolate | SRA ($cm^2/g$) | SRL (cm/g) | AvD (mm) | SRD ($cm^3/g$) |
|---|---|---|---|---|---|
| C1 | CONTROL | 847.29 ±44.46 a | 687.39 ±66.85 a | 0.40 ± 0.02a | 0.12 ± 0.00a |
| | 99 | 1027.44 ±130.77 a | 867.21 ± 151.79a | 0.39 ± 0.02 a | 0.11 ± 0.01a |
| | 128 | 1061.95 ± 146.42a | 881.21 ±66.73 a | 0.38 ± 0.03a | 0.11 ± 0.02a |
| | 135 | 1016.59 ±59.11 a | 882.89 ±91.54 a | 0.38 ± 0.02a | 0.11 ± 0.01a |
| | 141 | 1044.78 ± 68.45 a | 883.43 ±67.29 a | 0.38 ± 0.02 a | 0.10 ±0.01 a |
| | 152 | 914.74 ±50.78 a | 802.62 ± 69.77a | 0.37 ± 0.01a | 0.12 ± 0.00a |
| C2 | CONTROL | 1021.64 ±41.81 a | 909.61 ± 69.28a | 0.36 ± 0.01a | 0.11 ± 0.00 ab |
| | 99 | 1034.82 ±58.91 a | 922.61 ± 62.72a | 0.36 ± 0.01a | 0.11 ± 0.01ab |
| | 128 | 1217.28 ±204.39 a | 1220.43 ±310.45a | 0.35 ± 0.02a | 0.10 ±0.01ab |
| | 135 | 1239.66 ±109.67 a | 1056.51 ±116.53 a | 0.38 ± 0.02 a | 0.09 ± 0.01b |
| | 141 | 1284.28 ±207.40 a | 1214.20 ± 211.88a | 0.34 ± 0.01a | 0.10 ± 0.01b |
| | 152 | 917.84 ±26.31 a | 894.24 ± 32.09a | 0.33 ± 0.01a | 0.13 ± 0.00a |
| C5 | CONTROL | 881.00 ± 28.55 b | 609.24 ± 23.55b | 0.46 ± 0.01a | 0.10 ±0.00 ab |
| | 99 | 926.69 ±66.26 ab | 677.58 ± 47.12ab | 0.43 ± 0.01ab | 0.10 ±0.01 ab |
| | 128 | 1101.94 ± 96.50 a | 774.88 ± 72.32a | 0.46 ± 0.01a | 0.08 ± 0.01 b |
| | 135 | 841.07 ± 80.40 b | 645.94 ±60.04ab | 0.41 ± 0.01b | 0.12 ±0.01 a |
| | 141 | 767.82 ±44.72 b | 561.31 ± 32.04b | 0.44 ± 0.01ab | 0.12 ±0.01 a |
| | 152 | 786.94 ± 14.98 b | 605.28 ±18.08b | 0.42 ± 0.01b | 0.12 ± 0.00 a |
| C6 | CONTROL | 1337.83 ±665.09 a | 1121.95 ±531.20a | 0.38 ± 0.03ab | 0.17 ± 0.04a |
| | 99 | 707.81 ± 68.59 a | 553.35 ±62.87 a | 0.41 ± 0.01a | 0.14 ± 0.01a |
| | 128 | 633.89 ± 45.40 a | 524.96 ± 35.46a | 0.38 ± 0.01ab | 0.17 ±0.01 a |
| | 135 | 779.74 ± 53.88 a | 662.51 ±50.39 a | 0.38 ± 0.01ab | 0.14 ±0.01 a |
| | 141 | 1130.48 ±230.66 a | 1137.97 ± 325.22a | 0.34 ± 0.02b | 0.12 ±0.01 a |
| | 152 | 703.37 ± 79.40a | 622.32 ± 74.82a | 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 ($P<0.05$).

## Influence of synthetic SL analogue GR24 on bacterial motility

Regarding swimming motility, in isolate 99 (*K. radicincitans*) (Fig. 4-A), at 10 uM of acetone, presented a significant decrease in growth compared to the control with water. However, using other concentrations, bacterial colony growth was not significant different compared with GR24 treatment or control with water.

In isolate 135 (*B. tropica*) (Fig. 4-B), at 0.01 uM of GR24, presented a significant increase in growth compared to the control with acetone. However, using other concentrations, bacterial colony growth was not significant different compared to control with acetone or control with water.

In isolate 152 (*H. frisingense*) (Fig. 4-C), using any GR24 concentration, bacterial colony growth was not significant different compared to control with acetone or control with water. However, among treatments with GR24, there was a significant increase of the growth when 1uM of GR24 was applied compared to GR24 0.01uM and 10uM applications.

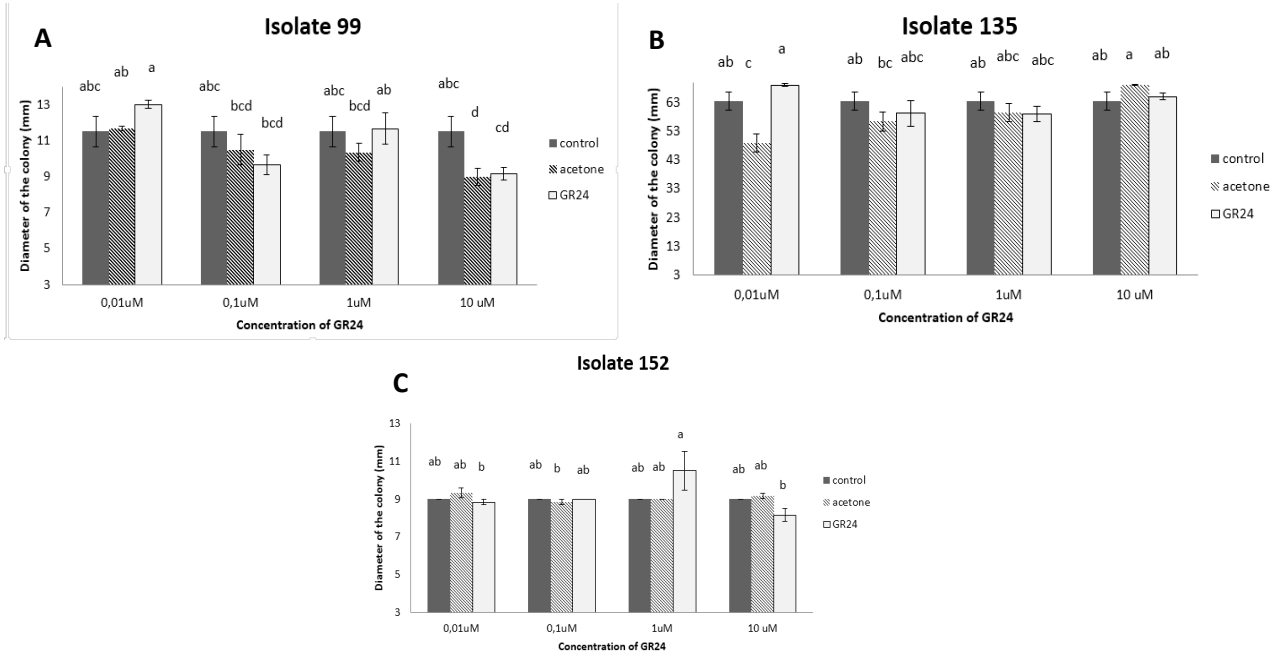


Fig 4: Swimming motility: bacterial isolates 99 (*K. radicincitans*) (A), 135 (*B. tropica*) (B), and 152 (*H. frisingense*) (C) growth in bacto media (BM) under different concentrations of GR24, acetone, and water (control). Measurements 2 days post inoculation (2dpi). Means of

replicates (n=6) ± (SE) followed by the same letter are not statistically different by Duncan test (P<0.05).

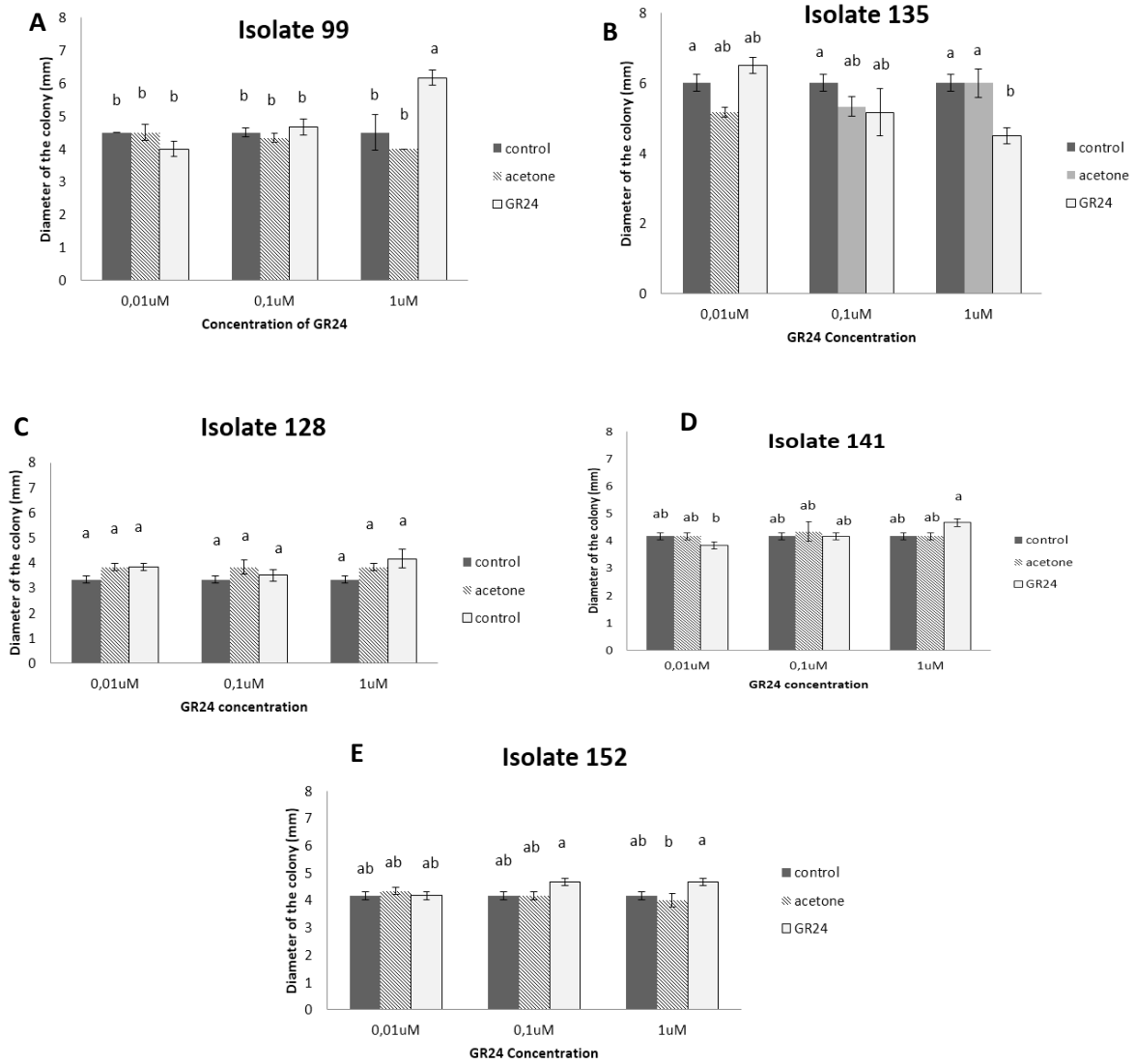


Fig 5: Surface motility: bacterial isolate 99 (*K. radicincitans*) (A) and 135 (*B. tropica*) (B) bacterial isolate 128 (*E. asburiae*) (C), 141(*P. fluorescens*) (D), and 152 (E) (*H. frisingense*) growth in minimal media (MM) under different concentrations of GR24, acetone, and water (control). Measurements 2 days post inoculation (2dpi). Means of replicates (n=6) ± (SE) followed by the same letter are not statistically different by Duncan test (P<0.05).

Regarding surface motility, isolate 99 (*K. radicincitans*) (Fig. 5-A), at 1uM of GR24, presented a significant increase in growth compared to the control with 1uM acetone and water control. However, using other concentrations, bacterial colony growth was not significant different compared with acetone treatment or control with water. Among treatments with GR24, there was a significant increase of the growth when 1uM of GR24 was applied.

In isolate 128 (*E. asburiae*) (Fig. 5-C), there were no significant changes in growth in any treatment comparing to the control acetone treatment or control with water.

In bacterial isolate 135 (*B. tropica*) (Fig. 5-B), 1uM of GR24, presented a significant decrease in growth compare to the control with 1uM acetone and water control. However, using other concentrations, bacterial colony growth was not significant different compared with acetone treatment or control with water. Among treatments with GR24, there was a significant decrease of the growth when 1uM of GR24 was applied.

In isolate 141 (*P. fluorescens*) (Fig. 5-D), there were no significant changes when using other concentration of GR24 and comparing to the acetone treatment or control with water. However, among treatments with GR24, there was a significant increase of the growth when 1uM of GR24 was applied compared with 0.01uM of GR24 application.

In isolate 152 (*H. frisingense*) test (Fig. 5-E), 1uM of GR24, presented a significant increase in growth compare to the control with 1uM acetone. However, using other concentrations, bacterial colony growth was not significant different compared with acetone treatment or control with water.

## Discussion:

In this work, we studied the inoculation effects of different sugarcane bacterial isolates on the growth and root architecture modification of different sorghum cultivar. Moreover, we evaluated the influence of GR24 SL molecule on the motility of the mentioned bacterial isolates.

Five bacterial isolates were chosen from a pool of PGPR isolated from sugarcane. Some studies agreed on the plant beneficial effect of bacterial isolates 99 (*K. radicincitans*), 128 (*E. asburiae*), 135 (*B. tropica*), 141 (*P. fluorescens*), and 152 (*H. frisingense*). For instance, *Burkholderia* species have nitrogen fixation activity in different plants, and can colonize internal tissues of the plant (Compant et al., 2008), which are ideal characteristics for PGPR. Moreover, it was mentioned that these species have been found colonizing maize plants, close related species of sorghum (da Silva et al., 2016). Regarding *Enterobacter* species, it was mentioned that *E. arbusiae* and *E. cloacae* have traits as nitrogen fixation activity, phytohormone production and phosphate solubilizing (Abraham & Silambarasan, 2015). Moreover, the isolate *H. cloacae* K7 has been found in sugar, which may support the hypothesis that some species, including the one we are using, can colonize sorghum roots (Kryuchkova et al., 2014).

Furthermore, a reason to use *Herbaspirillium* strain in this research was the ability of some species of this genus to influence in the root architecture and improve signalling pathways of plant hormone production (Straub et al., 2013). Furthermore, (2015) mentioned that *Kosakonia* promote growth in sorghum seedlings. Finally, some species of *Pseudomonas* genus were found colonizing roots of sorghum. They can have biocontrol activities as well as plant growth promoting activities (Funnell-Harris et al., 2013; Sajeli Begum et al., 2014).

Through different parameters, bacterial isolates *H. fringiensis* (152) and *B. tropica* (135) showed significant plant growth promoting effects. In corroboration with our results, Pereira et al. (2014) and da Silva et al. (2016) showed that the species *B. tropica* and *H. seropedicae* can promote growth in sugarcane and maize, which are also C4 grasses species as sorghum. Although these isolates showed some increase on sorghum biomass, this was not an evidence for all sorghum cultivars. It can be possible that bacterial species are specific to different plants. It may be possible because of physiological and genetic adaptation of microorganisms to the host plant (Requena et al., 1997).

Current results showed an increase in sorghum biomass when P limited plants were inoculated with *Herbaspirillium fringiensis* (152) we suspect that this isolate had been acting as P-solubilized agent. Studies from Estrada et al. (2013) support our findings, when they reported that *Herbaspirillium* can enhance plant yield by solubilizing calcium phosphate with is an insoluble form for plants. Furthermore, *Herbaspirillium* species have a high diversity in niches colonization, and can succeed easily (Straub, Yang, et al., 2013), which let us suspect that the increase on plant biomass could be related to the colonization of bacteria in the root tissues. However, further studies as expression of bacterial cells in the roots or in situ hybridization should be done. Therefore, bacterial colonization parameters may be correlated with plant growth effect.

It is known that *Burkholderia* species have endophytic and rhizospheric behaviour in sugarcane apart from nitrogen fixing and phosphate solubilizing activities, contributing to the growth of the plant (Estrada et al., 2013; Arnoldo, 2012; Reis et al., 2004). Interestingly, our results showed an increase of plant biomass in sorghum cultivars C1 and C5 inoculated with *B. tropica* (135). It is no possible to confirm that this influence was due to the effective inoculation or a side effect from the plant by the simple presence of these bacterial cells.

Furthermore, results showed that isolate *E. arbusiae* (128) influenced the increase of specific root length and area in sorghum cultivar C5. Such finding is in agreement the studies of Kryuchkova et al. (2014), who claimed that *Enterobacter* species can promote root length and lateral roots in sunflower.

Altogether showed that the bacterial isolates used in this experiment have certain effect on sorghum plants. It is worth to remind that those sorghum cultivars used in the experiment 1 were selected by different SL type and amount they produced. In this way, bacterial isolates could be reacted to SL compounds. That is why we decided to test them in vitro under the presence of SL analogue GR24.

Isolates 99 and 135 showed surface motility, depending the concentration of GR24 applied. However, whereas *K. radicincitans* (99) showed higher colonization in the petri dish in high concentration of GR24, *B. tropica* (135) restrained their growth in this condition. This could show possible of quorum sensing behaviour. (Quorum sensing (QS)) mediated by small molecules acyl-homoserine lactones (Pahlavan et al., 2012 ). QS could trigger the movement of the bacteria cells to specific ecological niches. (Venieraki et al., 2016). However, there are

not investigations regarding SL and quorum sensing in the literature. Thus, we believe that SL could work as a signal inducing or inhibiting the bacterial communication cell to cell.

Furthermore, it was mentioned by Straub et al., (2013), that *Herbaspirillium* species lack of production of acyl homoserine lactone, compound essential for quorum sensing. Thus, it could be the case for our results, where GR24 did not influence quorum sensing of isolate *H. fringiensis* (152) and subsequently not showing any effect on the colony spreading. Finally, bacterial isolates *E. asburiae* (128) and *P. fluorescens* (141) tested did not show significance when grown on the media, which let us think that other compounds regardless SL could be related with the motility in order to be attracted by plants.

As final remarks, it is shown that certain *B. tropica* (135) and *H. fringiensis* (152) have plant growth promotion effect in specific sorghum cultivars. Such cultivars produce different SL in different type and amounts. Moreover, the in vitro experiment showed that *B. tropica* (135) and *K. radicincitans* (99) motility were influenced by GR24. Then, SL secreted by P starved sorghum plants may be taking part of the influence on the bacterial movement, subsequently, isolates can interact actively with the sorghum plants. This mechanism would vary on the type of SL and species of bacteria isolate. Further investigations could be followed from the assumptions. Adjusting protocol of in vitro essays where the use of GR24 could influence the motility of bacterial isolates must be validates. Furthermore, testing other SL molecules, like orobanchol and 5- deoxystrigol, would be an interesting topic to investigate. Finally yet importantly, tests with RIL populations of sorghum crop should be carried on. In this way, it could provide us a clear insight of the interaction of SL and PGPR.

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