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Rhizobacteria inoculation and its effect on the productive parameters of sorghum

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ABSTRACT
Objective: To evaluate the effect of the *Bacillus megaterium* and *Pseudomonas japonica* rhizobacteria on the productive parameters of sorghum.
Design/Methodology/Approach: The experiment was carried out in Padilla, Tamaulipas, where the effect of inoculating a sorghum crop with the *Bacillus megaterium* KN13 and *Pseudomonas japonica* KC14 strains on its productive parameters was evaluated. Both strains were used in two concentrations (10^6 and 10^7 CFU). A randomized block design was applied, consisting of five treatments (two strains × two concentrations, plus a control), with six and nine replications.
Results: The *B. megaterium* and *P. japonica* strains can fix nitrogen and produce siderophores. Inoculating these strains into the sorghum crop increases grain yield, plant height, panicle length, plant stem diameter, and aerial dry weight. Better results are recorded when the strains have a 10^7 CFU concentration.
Study Limitations/Implications: Each type of soil and crop has various microbiomes.
Findings/Conclusions: The use of an adequate concentration of rhizobacteria improves sorghum production; therefore, it is a sustainable alternative, both for the nutrition of the crop and the reduction of the use of synthetic fertilizers.

Keywords: PGPR, *Bacillus megaterium*, *Pseudomonas japonica*.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most productive species and plays a major role in global food security (Mareya *et al.*, 2019). In Mexico, sorghum is the third most produced grain. In 2022, 1.4 million ha were planted nationwide; 55.8% of those hectares were located in Tamaulipas, which ranks first among the states that produce this species (SIAP, 2023). Sorghum is mainly used to produce food for the livestock sector. Synthetic fertilizers are applied to increase sorghum yield (and the yield of many other crops); however, high doses pollute and consequently impact soil fertility (Mikhak *et al.*, 2017).

The use of rhizobacteria is an option to replace synthetic fertilizers, reducing their negative impact on the soil and the environment (Vocciante *et al.*, 2022). Rhizobacteria promote plant growth, since they facilitate the absorption of nutrients (*e.g.*, nitrogen, phosphorus, and iron), as a consequence of their nitrogen fixation, phosphate solubilization, and siderophore production capacities (Haiyambo *et al.*, 2015). The genera *Pseudomonas* (Zahid *et al.*, 2015; Moreira *et al.*, 2016), *Bacillus* (Breedt *et al.*, 2017; Mumtaz *et al.*, 2017), *Azospirillun* (Florio *et al.*, 2017), and *Enterobacter* (Ibort *et al.*, 2017) stand out among the most studied plant growth-promoting rhizobacteria (PGPR).

The use of PGPR in various crops has been extensively researched. However, the lack of enough information on sorghum crops makes further studies into this matter a necessity. The objective of this work was to evaluate the effect of the *Bacillus megaterium* and *Pseudomonas japonica* rhizobacteria on productive parameters of sorghum.

MATERIALS AND METHODS

The experiment was carried out in 2020, at the ejido El Tablero (24° 05' N, 99° 00' W, and 36 m.a.s.l.), Padilla, Tamaulipas, Mexico. The physical and chemical characteristics of the sampling soil are: clay loam texture, high calcareous nature, low salts, pH=8.4, OM=2.9%, N=12.2 ppm, P=48.4 ppm, and K=364 ppm. The sampling was carried out at a depth of 0-30 cm.

Biological material

Two strains were isolated from the corn rhizosphere and identified as *Bacillus megaterium* KN13 and *Pseudomonas japonica* KC14. These strains have been evaluated in previous trials and are known to promote plant growth.

Nitrogen fixation evaluation

The indirect ammonium ion method was used to evaluate the ability of the two bacterial strains to fix nitrogen, using the Berthelot colorimetric technique (phenol-hypochlorite), as described by Escobar *et al.* (2011). A spectrophotometer was used for the absorbance reading (632.9 nm), considering all strains with positive readings as fixative. The evaluation was performed in triplicate for each strain.

Siderophore production

CAS agar was used to determine if both strains produced siderophores (iron chelating compounds). After culturing the bacteria at 30 °C for 5 days, the presence of orange halos was detected around the colony, indicating siderophore activity. For each strain, the assay was performed in triplicate (Haiyambo *et al.*, 2015).

Field trial

Before sowing, the seeds of sorghum hybrid D47 were sterilized (5 min in 70% ethanol, 5 min in 10% NaClO, and three rinses with sterile distilled water) and kept at 4 °C for 24 h (Ibort *et al.*, 2017). The two strains were allowed to grown in LB for 24 h at 28 °C. They were stirred at 200 rpm. CFU were counted with a Neubauer chamber. The bacterial solutions were adjusted to a concentration of 10^6 and 10^7 CFU mL^{-1}. Subsequently, the seeds were inoculated via a 1-h immersion in each of the bacterial solutions. A second inoculation was performed 25 days after sowing, adding 2.5 mL of bacterial solution per plant. The same treatment was carried out on the control plants, using sterile distilled water instead of the bacterial solution (Angulo *et al.*, 2014). Agronomic management consisted of two relief irrigations, mechanical weed control, pest control (with chemical insecticides), and no fertilization. The harvest was carried out 135 days after sowing.

Experimental design

A completely randomized block design was used. The experiment consisted of five treatments, with six repetitions for the performance variables and nine repetitions for the morphometric variables. The treatments were called Bm7, Bm6, Pj7, and Pj6 (Bm: *Bacillus megaterium* KN13; Pj: *Pseudomonas japonica* KC14; 7: 10^7 CFU; and 6: 10^6 CFU). The variables evaluated were: furrow grain weight, grain yield (ha^{-1}), plant height, panicle length, plant stem diameter, and aerial dry weight. To calculate biomass, plants were dried in an oven at 70 °C for 48 h. Rows (7 m long, 0.8 m between rows) were established with a population density of 20 plants per linear m. To calculate the yield, 5 linear m of each repetition were harvested, projecting the production in kg ha^{-1}.

Statistical analysis

A general linear model analysis and multiple comparison of LSD means test (significance level: $P \leq 0.05$) were performed using the Rstudio statistical software, version 4.0.3.

RESULTS AND DISCUSSION

Table 1 shows the results of the biochemical tests. Both strains can fix nitrogen and produce siderophores; therefore, they can improve the availability of nitrogen and iron and suppress the effect of phytopathogens (Beneduzi *et al.*, 2012). In other words, they are rhizobacteria with the potential to promote plant growth (Haiyambo *et al.*, 2015). Specifically, *B. megaterium* has nitrogen fixation abilities (Yousuf *et al.*, 2017). The siderophores secreted by microorganisms are usually the source of iron for sorghum cultivation (Beneduzi *et al.*, 2012).

Figure 1 shows the results for sorghum grain yield, including a highly significant statistical difference between treatments regarding furrow grain weight (F=60.02, g.l.=4, P<4.752e-09***) and grain yield in kg ha^{-1} (F=60.17, g.l.=4, P<4.668e-09***). There

Table 1. Evaluation of the ability of rhizobacteria to nitrogen fixation and siderophore production.

Strains	Biochemical tests	
	Nitrogen fixation	Siderophore production
Bm KN13	+	+
Pj KC14	+	+

Bm KN14=*Bacillus megaterium*. Pj KC14=*Pseudomonas japonica*.

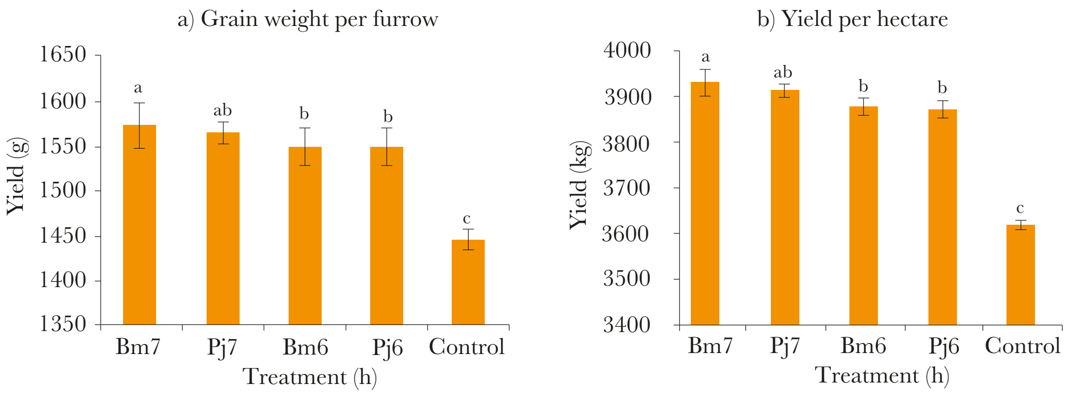

Figure 1. Effect of rhizobacteria inoculation on sorghum grain yield. Bm7=*B. megaterium* (10^7 CFU). Bm6=*B. megaterium* (10^6 CFU). Pj7=*P. japonica* (10^7 CFU). Pj6=*P. japonica* (10^6 CFU).

were no statistical differences between these two variables, neither for the block, nor for the block × treatment interaction. The treatment inoculated with Bm7 obtained the highest yield, while the control reported the lowest yield. Previous studies report increases in grain production in wheat (*Triticum* spp.) inoculated with *Bacillus* spp. (Upadhyay and Singh, 2015) and *Pseudomonas* spp. (Nadeem *et al.*, 2013). The same effect has been reported for corn inoculated with *Bacillus* and *Pseudomonas* spp. (Moreira *et al.*, 2016, Mumtaz *et al.*, 2017). The 10^7-concentration obtained higher results than the 10^6-concentration. João-Alves *et al.* (2021) reported that, in a study with corn and soybean, increasing the concentration of the bacterial solution increased production. In conclusion, a higher concentration of CFUs in these strains is necessary to obtain better results.

The results of the morphometric variables are presented in Table 2. Regarding the plant height, aerial dry weight, and stem diameter variables, only the following treatments recorded a statistical difference: F=31.1676, g.l.=4, P<3.465e-09***; F=67.74, g.l.=4, P<1.012e-112***; and F=5.2862, g.l.=4, P<0.003374**. The block and the interaction do not show a statistical difference. Regarding the panicle length variable, the following treatments had a statistical difference: F=70.2913, d.f.=4, P<6.75e-13*** and the block F=7.7873, d.f.=2, P<0.0002475***. The one exception was the block × treatment interaction.

Table 2. Effect of rhizobacteria inoculation on the morphometric variables of sorghum.

Treatment	Plant height (cm)	Panicle length (cm)	Aerial dry weight (g)	Stem diameter (cm)
Pj7	122±0a	22.8±0a	124±0a	24.5±0a
Bm7	121.22±1.86a	23.21±0.28a	124.78±2.86a	24.78±1.09a
Pj6	120.67±1.41a	22.98±0.186a	122.78±2.64a	24±1.22a
Bm6	120.56±1.67a	23±0.353a	123.11±2.80a	24.78±0.83a
Control	114±1.5b	21.48±0.399b	106.33±2b	23±0.86ab

Bm7=*B. megaterium* (10^7 CFU). Bm6=*B. megaterium* (10^6 CFU). Pj7=*P. japonica* (10^7 CFU). Pj6=*P. japonica* (10^6 CFU).

Compared to the control plants, the plants inoculated with both concentrations of the two strains had a positive effect on the evaluated parameters. Similar results have been reported in corn plants, where the use of *Bacillus* spp. and *Pseudomonas* spp. had a positive effect: it promoted growth and increased forage and grain production (Nadeem *et al.*, 2013; Zahid *et al.*, 2015; Mumtaz *et al.*, 2017). Positive effects have also been reported on the biomass production of wheat crops inoculated with *Bacillus* spp. and *Pseudomonas* spp. (Upadhyay *et al.*, 2011; Nadeem *et al.*, 2013; Upadhyay and Singh, 2015). This study only evaluated the capacity of the strains to fix nitrogen and produce siderophores; however, these two genera of bacteria can promote growth by other mechanisms, such as phytohormone production and the solubilization of phosphate, potassium, and zinc (Vaikuntapu *et al.*, 2014; Gandhi and Muralidharan, 2016; Mumtaz *et al.*, 2017), as well as the release of some micronutrients (Haiyambo *et al.*, 2015). The effect of *B. megaterium* and *P. japonica* on the growth promotion and production of sorghum may be influenced by a wide range of the mechanisms of action that are attributed to these bacteria.

CONCLUSIONS

Taking into consideration the trend towards the reduction in the use of synthetic fertilizers, the use of rhizobacteria to improve sorghum production is a feasible and environmentally acceptable alternative. The results show that a 10^7 CFU concentration of rhizobacteria improves sorghum production. Further studies are required to evaluate the optimal number of inoculations, as well as the specific concentration at which each strain records the best results.

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