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Effect of *Bacillus* spp. on the germination and growth of roselle plants (*Hibiscus sabdariffa* L.)

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ABSTRACT

Objective: To analyze the effect of three native strains of *Bacillus* spp. on roselle (*Hibiscus sabdariffa* L.) germination and plant growth of the tecoanapa variety in greenhouse conditions using the Bio-priming method.

Design/methodology/approach: The identity of the Bacillus strains was verified using PCR technique with the universal primers 27F and 1492R for the amplification of the 16S rDNA gene. The roselle seeds were treated with bacterial cells of *Bacillus* spp. with Bio-priming method, evaluating the effect on germination and plant growth. The percentage of germination was evaluated, as well as plant height, root length, and dry matter of plants and roots.

Results: Molecular identification of *B. velezensis* (T1), *B. amyloliquefaciens* (T2), and *B. subtilis* (T3) was carried out. The three treatments caused an increase in germination percentage, root length and plant height, and there was also an increase in dry matter weight of plants and roots, with a significant difference between treatments 1, 2, 3 and the control.

Study limitations/implications: Strains of *Bacillus* spp. must reach commercial production for field applications.

Findings/conclusions: *B. velezensis* is the species that demonstrated the highest percentage of germination and a growth-promoting effect, followed by *B. amyloliquefaciens* and *B. subtilis* respectively.

Key words: Bio-priming, seed, growth

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) (Malvaceae) also known as Jamaica rose, Abyssinian rose or Jamaica flower, originated in tropical Africa, although it is now cultivated in Mexico, Central and South America and Southeast Asia (Morton, 1987; Sayago and Goñi, 2010). Their calyces are the most useful and of socioeconomic importance, since they are used to obtain extracts with applications in the pharmaceutical and food industries (Galicia *et al*., 2008).

In Mexico presently 18,654 ha of roselle are grown, with the state of Guerrero occupying first place as producer with 76% of that surface. The plant grows better in regions with tropical and subtropical climates in the municipalities of Tecoanapa, Ayutla de Los Libres, Acapulco de Juárez, San Luis Acatlán, Juan R. Escudero, and San Marcos (SIAP, 2019).

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There is a recent demand in production for the development of new systems aimed at a lower environmental impact. The genus *Bacillus* is considered a plant growth-promoting bacteria (PGPB), and contributes benefits to plants since it has the capacity to exert a positive effect on plant growth by various mechanisms including the production of phytohormones, solubilization of phosphate, nitrogen fixation and biological control of pathogens (Lugtenberg and Kamilova, 2009). Based on this, this study had the objective of analyzing the effect of three native strains of *Bacillus* spp. on the germination and plant growth of the tecoanapa roselle variety (*Hibiscus sabdariffa* L.) in greenhouse conditions, using the Bio-priming method, which consists of coating the seeds with the bacterial agent in conjunction with a hydration process before sowing.

MATERIALS AND METHODS

Bioassays were carried out with three *Bacillus* isolates obtained from the collection at the Bacteriological Laboratory of Colegio de Postgraduados. The strains are native to roselle cultures, isolated from calyces and previously identified as *B. subtilis*, *B. amyloliquefaciens* and *Bacillus* sp. (Rendón, 2014). For the inoculations the tecoanapa roselle cultivar was used.

Molecular identification of *Bacillus*

The identity of the three strains of *Bacillus* was verified using PCR technique. The DNA extractions were carried out using the commercial kit Wizard Genomic DNA Purification, following the supplier's instructions. The universal primers used for the amplification of the 16S rDNA gene were 27F (5´AGAGTTTGATCATGGCTCAG) / 1492R (5´GGTTACCTTGTTACGACTT) (Lane, 1991; Turner *et al*., 1999). The amplifications were achieved with an Eppendorf Nexus thermocycler with the following program: a denaturalization cycle for 3 min at 94 °C, followed by 25 three-step cycles: denaturalization for 30 s at 94 °C, 30 s annealing at a recognition temperature for each primer pair, and an extension cycle for 1 min at 72 °C. The PCR amplifications were analyzed by electrophoresis in agarose gel. The PCR products were sent in for sequencing to Macrogen Korea (http://www.macrogen.com/eng/). The consensus sequence was submitted for alignment and homology analysis with the bioinformatic tool BLAST (Basic Local Alignment Search Tool) in GenBank from NCBI (https://www.ncbi.nlm.nih.gov/genbank).

Inoculation of *Bacillus* in roselle seeds

The seed treatment was carried out using the Bio-priming method (Rivera *et al*., 2017) with some modifications. A suspension of *Bacillus* cells was used at a concentration of 108 CFU/ml, obtained from nutritional agar plates after 2 d of growth. The effect of the inoculation with bacteria was compared with seeds treated with distilled water. Four treatments were established (T1: *B. subtilis*; T2: *B. amyloliquefaciens*; T3: *Bacillus* sp.; T4: control).

Effect of *Bacillus* on germination and growth of roselle plants

The treated seeds were sown in plastic trays with 200 cavities with peat moss (European Kekkila Peat moss) sterilized at 121 °C for 1.5 h. The trays were stored in greenhouses, where daily germinated seed counts were carried out. Thirty days after sowing, the plants were transplanted to 30 × 30 cm black polyethylene bags with peat moss (European Kekkila peat moss) and organic material (sheep manure) in a 90:10 ratio respectively. The variables evaluated were: germination percentage, plant height, root length and dry matter in plants and roots.

RESULTS AND DISCUSSION

Molecular identification of *Bacillus*

The results of the consensus submitted for homology analysis with BLAST demonstrated identity percentages above 99% for all of the samples when compared with the NCBI database. The preliminary identification of *B. subtilis* was corrected for *Bacillus velezensis* and it was verified that the second strain corresponded to *B. amyloliquefaciens*. The third strain determined for genus showed a 100% match with *B. subtilis* for the consensus sequences of primers 27F and 1492R (Table 1).

Table 1. BLAST analysis results.

Variable	Primers	Result BLAST	Accession	Percent identify
B. subtilis	27F/1492R	*Bacillus velezensis*	MT538583.1	100.00%
B. amyloliquefaciens		*Bacillus amyloliquefaciens*	JF802170.1	99.05%
Bacillus sp.		*Bacillus subtilis*	MN417010.1	100.00%

Effect on germination

The first seeds to sprout were those treated with *B. velezensis* (T1), *B. amyloliquefaciens* (T2) and *B. subtilis* (T3), and by the fourth day after sowing they reached 80% of total germination. The control (T4) at day six had not initiated germination,

suggesting that the bacterial inoculum accelerated the germination process of the seeds. T1 was the best with 95.63% of seeds germinated, followed by T2 with 94.38%, T3 with 93.75%, and T4 with 80%. Final germination was reached 8 d after sowing for T1, T2, and T3, and 15 d later for T4. This coincides with what was reported by Mojica *et al*. (2009), who described an increase in germination percentage with inoculation treatment with *B. thuringiensis* on *R. solani*.

Growth-promoting effect

It was demonstrated that T1, T2 and T3 promoted an increase in root length and plant height, as well as an increase in dry matter weight of plants and roots, with a significant difference between treatments 1, 2, 3, and the control (T4) (Table 2). Previous studies have reported the *Bacillus* genus as bacteria that are beneficial to improve plant growth (Rojas *et al*., 2016).

The results showed that *B. velezensis* is the species with the most potential as a growth promoter, followed by *B. amyloliquefaciens* and finally *B. subtilis* (Figure 2). Recently there have been reports on many strains of *Bacillus* which promote plant growth and monitor biocontrol, including *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus velezensis* (Wang *et al*., 2020).

The *Bacillus* genus is one of the most studied due to its capacity to promote plant growth of important crops (Rojas *et al*., 2011; Hernández *et al*., 2014; Cabra *et al*., 2017).

Recent Bio-priming studies, dealing with mechanisms in the use of plant growth-promoting bacteria, have described *Bacillus* strains with the capacity to produce auxins that stimulate plant growth and phosphate solubilization (Tejera *et al*., 2012). Together with this study's results, they suggest a plant-bacteria interaction that improves plant development. The results obtained in the greenhouse study reveal that the bacteria evaluated manifested growth promoting activity. The three strains of *Bacillus* belong to the plant growth-promoting bacteria (PGPB) rhizobacteria group (Wang *et al*., 2020).

CONCLUSIONS

B. velezensis is the species that showed the highest germination percentage and growth-promoting effect, followed by *B. amyloliquefaciens* and then *B. subtilis*.

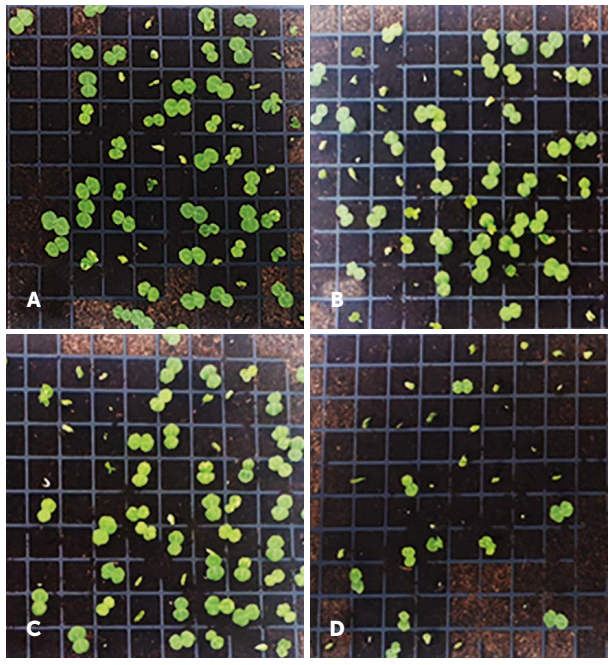

Figure 1. Germination of *Hibiscus sabdariffa* L. seeds eight days after sowing and inoculated with: A) T1: *B. velezensis*. B) T2: *B. amyloliquefaciens*. C) T3: *B. subtilis*. D) T4: control.

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Table 2. Statistical values of treatment for growth variables of *Hibiscus sabdariffa* L. plants inoculated with bacteria.

Treatment	AP	PSP	LR	PSR
T1: *Bacillus velezensi*	38.48 a	5.57 a	31.83 a	0.912 a
T2: *Bacillus amyloliquefaciens*	37.07 a	5.21 ab	27.60 b	0.738 b
T3: *Bacillus subtilis*	34.93 b	4.77 b	26.33 b	0.707 b
T4: control	30.67 c	2.71 c	16.20 c	0.264 c
DMSH	1.84	0.66	3.91	0.110

AP Plant height; PSP Plant dry matter; LR Root length; PSR Root dry matter (All initials based on Spanish terms).

Figure 2. Differences in plant height, root length, and root volume of *Hibiscus sabdariffa* L. inoculated with A) T1: *B. velezensis*. B) T2: *B. amyloliquefaciens*. C) T3: *B. subtilis*. D) T4: control.

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