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Plant growth-promoting rhizobacteria in tomato seedling production

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Abstract

Plant growth-promoting rhizobacteria represent a biotechnological alternative to improve the production of socio-economically important species such as tomato (*Solanum lycopersicum* L.). The production of high-quality and safe seedlings is a key challenge prior to field establishment. One of the main obstacles for farmers are the high costs associated with sanitary inputs and fertilizers necessary to promote good rooting and reduce plant mortality. The objective of this research was to isolate rhizobacteria from the avocado rhizosphere with the capacity to produce indoleacetic acid (IAA) and evaluate their effect on root development of tomato seedlings *in vitro*. Two IAA-producing strains were evaluated and inoculated on seeds of two tomato genotypes (H13-37 and L3), grown *in vitro* using Murashige Skoog medium. The experiment was carried out in a completely randomized block design two weeks after sowing. The isolated bacterial strains produced sufficient IAA to promote root development. The L3 genotype had the best response regarding root length, total surface area, and number of branches due to bacterial inoculation.

Keywords: *Solanum lycopersicum* L., indoleacetic acid, IAA, PGPR, growth promoters.

Introduction

Tomato production (*Solanum lycopersicum* L.) in Mexico holds significant importance, not only due to its high consumption but also because of its nutritional properties. In 2022, national production reached 3.46 million tons (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2023). As a result, there is growing interest in improving profitability and sustainability of tomato crops to ensure both per capita supply and the preservation of natural resources. The use of microorganisms as biofertilizers has emerged as an effective strategy to reduce the environmental impact of agrochemicals and promote more sustainable agricultural practices (Ahamad et al., 2023). Among these microorganisms used as biofertilizers are plant growth-promoting rhizobacteria (PGPR), which belong to different bacterial species capable of enhancing plant growth and productivity (Saeed et al., 2021).

Rhizobacteria play important roles such as promoting and facilitating plant growth and development through direct mechanisms by producing metabolites, such as indoleacetic acid (IAA), phosphate solubilization, siderophore production, biological nitrogen fixation (BNF), and 1-aminocyclopropane-1-carboxylic acid (ACC) production. Also, these organisms act through indirect mechanisms such as synthesis of ACC deaminase and siderophores, induction of systemic resistance, and antagonism against pathogenic fungi and bacteria (Mohanty et al., 2021; Orozco-Mosqueda et al., 2018).

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Native PGPRs represent a viable alternative both economically and ecologically in agriculture, as their versatility and ability to produce plant growth regulators contribute to improving the productivity of economically important crops like tomatoes. However, some tomato producers, such as those in San Juan Elotepec, Sola de Vega, Oaxaca, face significant challenges in acquiring seedlings for transplanting, which increases production costs. Although they have opted to produce their own seedlings, improper management and inadequate nutrition after germination negatively impact root system development, leading to seedling losses in the field and increased expenses on rooting agents and agrochemicals.

Therefore, the objective of this study was to isolate PGPR from the avocado rhizosphere with the capacity to produce IAA and to evaluate their effect on the root development of tomato seedlings *in vitro*.

Materials and methods

Physical and chemical soil characterization

A total of seven soil subsamples were collected at a depth of 0-15 cm from the rhizosphere of a 'Hass' avocado orchard in San Juan Elotepec, Sola de Vega, Oaxaca, Mexico. From them, a composite sample was formed for characterization according to the guidelines of NOM-021-SEMARNAT-2000 (Diario Oficial de la Federación [DOF], 2000), which establishes the specifications for the analysis of fertility, salinity and soil classification, as well as survey and sampling procedures. Soil characteristics are shown in Table 1.

Collection and selection of IAA-producing bacterial strains

Isolation and purification of rhizobacteria was performed using the procedure indicated by Zúñiga-Dávila (2012). For this purpose, a series of serial decimal dilutions of soil samples were prepared with isotonic saline solution (ISS) until reaching a concentration of 10^{-6} g·mL^{-1}. Subsequently, 1 mL of the 10^{-5} and 10^{-6} dilutions was transferred in triplicate to sterile Petri dishes and tryptone glucose extract (ATGE) agar was added. The soil aliquot and culture medium were gently mixed, solidified, and incubated at 28 °C for 48 h. Seven colonies with different macroscopic morphology were selected and reseeded by streaking on ATGE plate until pure cultures were obtained

IAA production was assessed by the colorimetric method for auxin detection (Ahmad et al., 2005). Briefly, suspensions of each selected strain were prepared at a concentration of 300×10^{6} cells·mL^{-1} (equivalent to #1 on the McFarland scale) in sterile ISS. From each suspension, 1 mL was transferred, in triplicate, to tubes containing tryptone glucose extract (TGE) agar enriched with L-tryptophan (5 mM), which were incubated at 28 °C for 5 d. Subsequently, 1 mL of each culture was mixed with 4 mL of Salkowsky's reagent and, after 30 min of incubation in the dark, absorbance was measured at 530 nm in a visible light spectrophotometer (Genesys™ 20, USA). Simultaneously, a calibration curve was prepared with commercial IAA (0, 10, 20, 20, 40 and 60 µg·mL^{-1}). The absorbance values were interpolated on the calibration curve (Figure 1a) to determine the concentrations of IAA produced by each strain (Figure 1b).

Table 1. Physical and chemical characteristics of the soil.

Parameters	Value	Qualitative interpretation*
Texture	-	Clay loam
pH	7.6	Medium alkaline
Bulk density (g·cm^{-3})	1.2	Mineral soil
Organic matter (%)	0.4	Very low
Total nitrogen (%)	0.04	Very low
Usable phosphorus (mg·kg^{-1})	5.1	Low
Potassium (mg·kg^{-1})	170	Moderate-low
Calcium (mg·kg^{-1})	6400	Very high
Magnesium (mg·kg^{-1})	5876	Very high
Manganese (mg·kg^{-1})	0.65	Poor
Copper (mg·kg^{-1})	0.12	Poor
Zinc (mg·kg^{-1})	0.41	Poor
Iron (mg·kg^{-1})	1.1	Poor

* Classification according to the criteria of NOM-021-SEMARNAT-2000 (DOF, 2000).

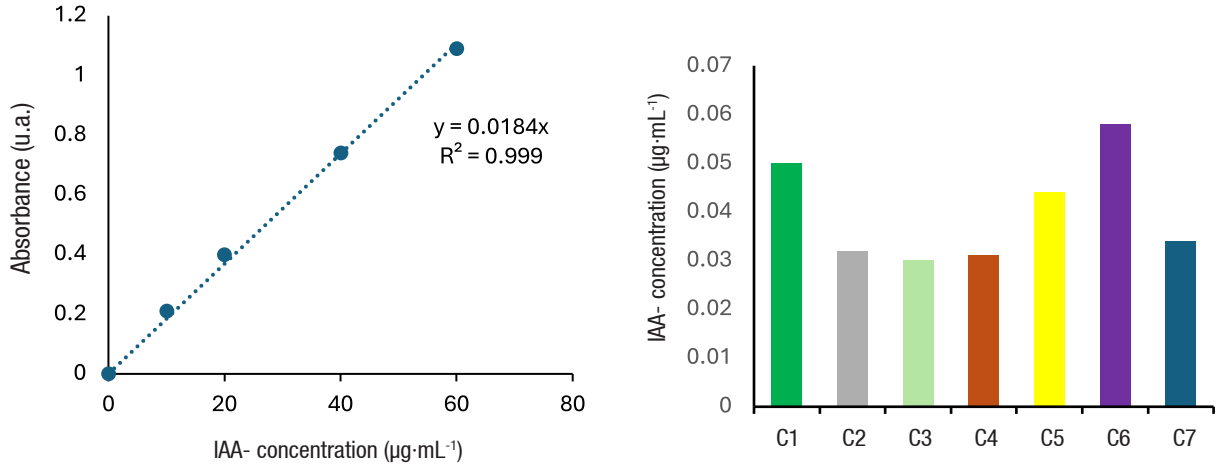

Figure 1. Detection of concentration of indoleacetic acid (IAA). Left: calibration curve. Right: IAA production by bacterial strains.

Application of treatments and experimental design

The strains with the highest IAA production capacity (C1 and C6) were propagated for 5 days in TGE broth at 28 °C with constant agitation at 100 rpm and adjusted to a concentration equivalent to 300-350×10^6 cells · mL^{-1} of the McFarland scale. A mixture of both strains (C1C6) and a control treatment with ISS (C0) were prepared. The experiment was set up *in vitro* in test tubes with Murashige and Skoog (MS) medium and seeds of two tomato genotypes (G1=H13-37 and G2=L3), donated by Dr. Juan Enrique Rodríguez Pérez from the Universidad Autónoma Chapingo.

The experimental design was a completely randomized block design (CRBD), where the blocks corresponded to the genotypes and the treatments (C0, C1, C6, C1C6), all evaluated at one concentration level with 10 repetitions per treatment. The experimental unit consisted of one seed per test tube. For this, the seeds were surface disinfected with a 20 % sodium hypochlorite solution. Subsequently, immersed in 1 mL of the bacterial suspension corresponding to each treatment for 12 hours. Finally, planted in test tubes containing MS medium. The experimental units were placed in an area illuminated with LED light at 1,000 lux, and the basal part of the tubes was covered to ensure complete darkness.

Treatment evaluation

The evaluation was carried out two weeks after planting. The variables evaluated were root length (cm), surface area (cm^2), root volume (mm^3) and branching (number of secondary roots) using the root image analysis system Winrhizo® 2016. For data analysis, the R programming language and the R environment version 4.2.1 for Windows were used. The values obtained were subjected to Tukey's comparison of means ($P \leq 0.05$). Homogeneity and normality of residuals were evaluated using Levene's test and Shapiro-Wilk test, respectively.

Results and discussion

Inoculation with IAA-producing rhizobacteria showed favorable effects on the two tomato genotypes evaluated (G1=H13-37 and G2=L3), in relation to the root parameters analyzed (Figure 2). In G1, strain C1 stood out by significantly increasing root length compared to the control (C0). In contrast, the combination of strains (C1C6) showed no significant differences compared to the control in this same parameter. On the other hand, in G2, strains C1C6 and C6 generated better results, C6 was the strain that showed the greatest effect on root length (Figure 2a).

In root length distribution (Figure 3), the different strains favored distributions in lengths from 0 to 0.5 cm, while the treatment without bacterial inoculation (C0) was characterized by having a main root and low percentage of root formation. Both genotypes responded to the IAA stimulation produced by the inoculated strains.

Regarding root volume (Figure 2b), the combination of strains had a significant effect on volume increase compared to the control, where G1 was the most important. In contrast, individual strains had a low impact, similar to the control in both genotypes. Regarding surface area (Figure 2c), G2 showed greater area with the inoculation of both strains, followed by inoculation with C6 and C1, compared to the control. In G1, strain C1 was the one that showed the greatest increase in surface area, followed by strains C6 and C1C6, in relation to the control.

For the number of branches (Figure 2d), G2 showed significantly more branches with inoculation of strain C6,

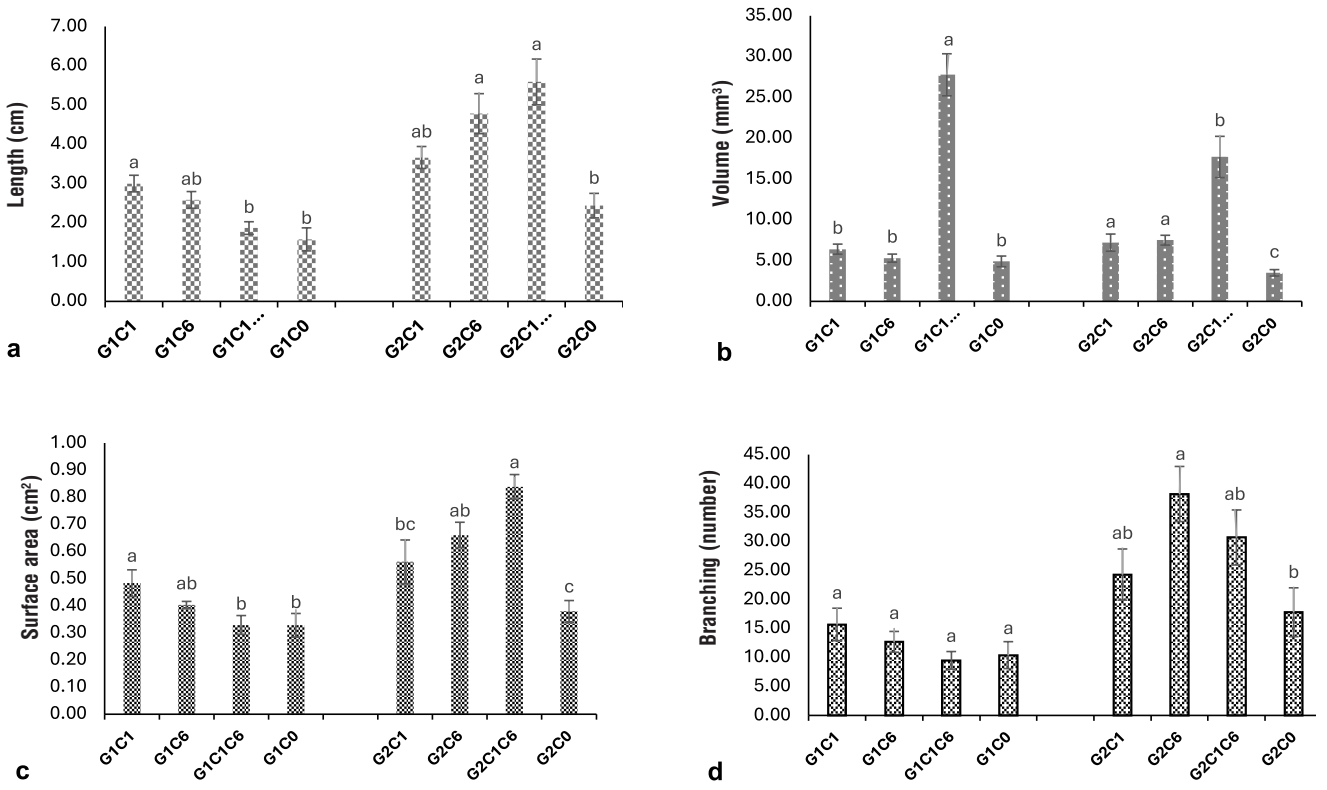

Figure 2. Root parameters obtained in the two tomato genotypes (G1=H13-37 and G2=L3) inoculated with different strains (C1, C6, C1C6 and C0): a) length, b) volume, c) surface area and d) seedling branching. Different letters in each column indicate statistical differences (Tukey, $P \leq 0.05$).

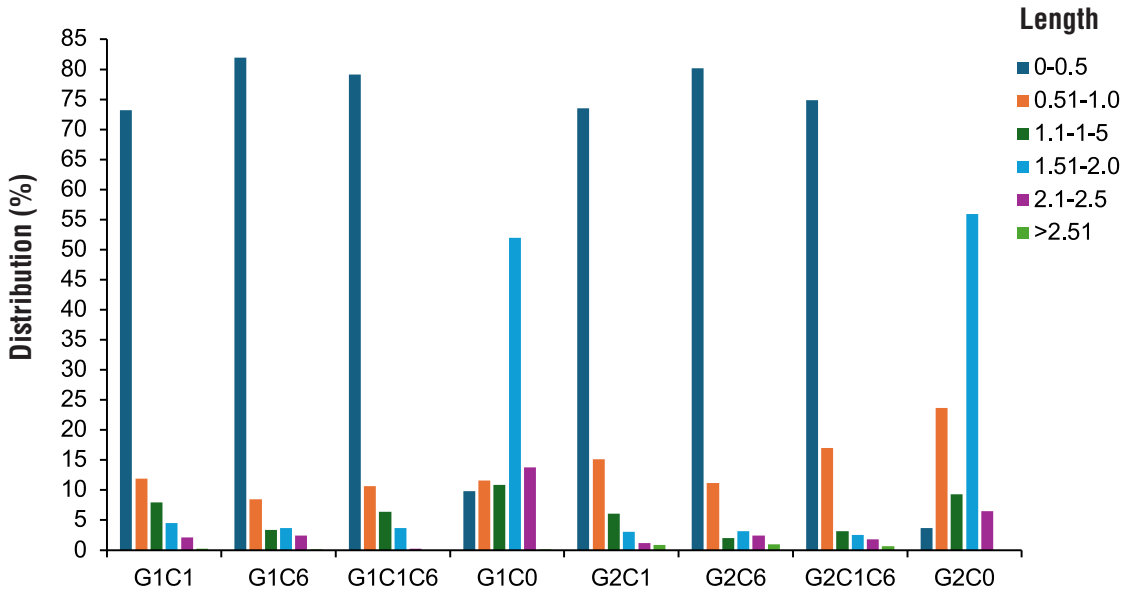

Figure 3. Root length distribution in seedlings of two tomato genotypes (G1=H13-37 and G2=L3) inoculated with bacterial strains (C1, C6, C1C6 and C0).

followed by C1C6 and C1, compared to the control. On the other hand, G1 showed no significant statistical difference in the number of branches.

The concentration of IAA needed to stimulate plant root growth depends on the species. A particular genotype may be favored by one strain at a certain concentration, but the same concentration may not have a significant influence on another genotype. In this regard, bacteria-plant interaction promotes plant growth through the production of phytohormones such as IAA (Sharma et al., 2021), which contributes to the development of the root system (Chauhan et al., 2013). The results obtained in this study agreed with that reported by Irizarry and White (2017), who mention that rhizobacteria induce structural modifications in root architecture. Kumar et al. (2019), reported the development and elongation of root hairs. Good root development provides better anchorage, greater water and nutrient uptake, and increased exploration of the surrounding environment, which can reduce the use of chemical fertilizers (Khan et al., 2021).

The results also coincide with those mentioned by Moreno-Gavíra et al. (2020), who reported an increase of 18.23 % in root length in tomato seed germination compared to their control treatment, and 17.85 % in bell pepper seeds inoculated with *P. variotii* (IAA-producing strain). Asari et al. (2017) observed changes in root architecture in *Arabidopsis* by rhizobacterial inoculation, and Garcia et al. (2021) observed something similar in *Glycine max* L.

Conclusions

Strains of plant growth-promoting bacteria with the ability to produce indoleacetic acid (IAA) were isolated. The IAA production capacity was sufficient to significantly differentiate the effect of the treatments on both evaluated genotypes. Genotype 2 (L3) showed a remarkable response to the inoculation of IAA-producing bacteria, with improvements in the length, surface area, and number of branches in the root system. The rhizobacteria proved to be an effective strategy for promoting and optimizing the growth of tomato seedlings, suggesting their potential as biofertilizers in sustainable agricultural systems.

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