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<Modulating the PGPR activity of *Lysinibacillus pinottii* sp. nov. PB211 through plant sensitivity/resistance to exogenous auxins

Modulación de la actividad PGPR de *Lysinibacillus pinottii* sp. nov. PB211 a través de la sensibilidad/resistencia de las plantas a las auxinas exógenas

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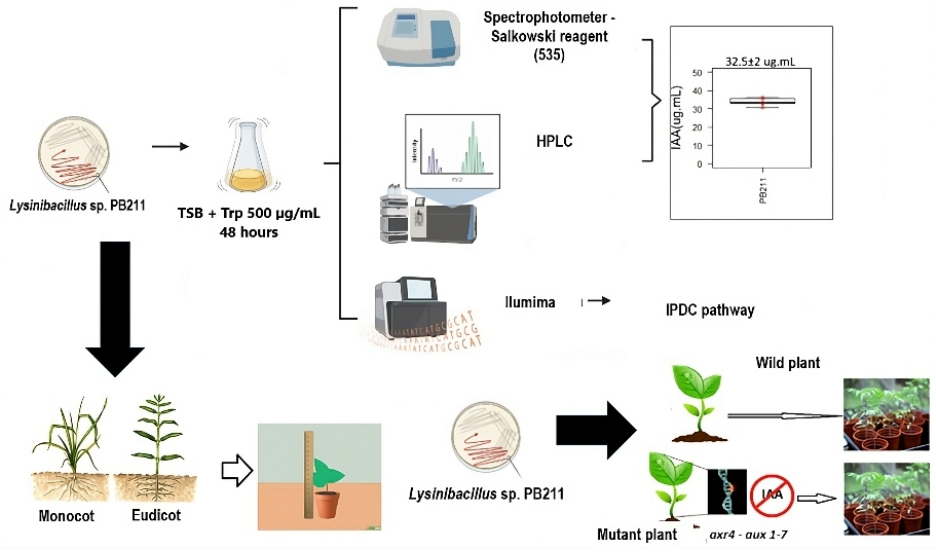

Modulating PGPR activity via plant auxin sensitivity. Photo: M. Pantoja-Guerra

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ABSTRACT

The effectiveness of plant growth-promoting rhizobacteria (PGPR) strains indole-3-acetic acid (IAA) producers may be influenced by the plant's resistance to auxins. In this work, the impact of auxin resistance on the PGPR activity of *Lysinibacillus pinottii* sp. nov. PB211 was investigated. PB211 produced an average of 32 µg mL^{-1} of indole-3-acetic acid (IAA). Genetic evidence indicated that PB211 utilizes the indole pyruvic acid pathway for IAA synthesis. Regards to the response of plant models to IAA treatment, eudicot models (cucumber and bean) exhibited higher sensitivity to IAA compared to monocot models (corn and brachiaria). Monocots required higher IAA concentrations to elicit phenotypic changes in root architecture. The resistance/sensitivity of plants to exogenous auxins was found to modulate the PGPR activity of PB211. Inoculation with PB211 at varying concentrations resulted in differential effects on plant models. Eudicots displayed significant PGPR activity from lower inoculum concentrations, whereas monocots required higher inoculum concentrations to exhibit a similar consistent effect. The PB211 effect also was evaluated on *Arabidopsis thaliana* wild-type (col-0 "auxin-sensible") and mutant (aux1-7/axr4-2 "auxin-resistant") plants. PB211 had a "bell-shaped" effect on wild-type plants response, a typical response of auxin-activity, so, the PGPR effect decreased at the highest inoculum concentration. Conversely, the mutant plants exhibited increased PGPR activity with higher inoculum concentrations, compensating for their auxin-deficient phenotype. These findings suggest that the resistance/sensitivity of plants to exogenous auxins influences the effect of auxin-producer PGPRs. These relationships could facilitate the development and application of more effective biological inoculants for agriculture.

Additional key words: monocots; eudicots; indole-3-acetic acid; plant growth promotion; biofertilizers; biostimulants.

RESUMEN

La efectividad de las cepas de rizobacterias promotoras del crecimiento vegetal (PGPR) productoras de ácido indol-3-acético (AIA) puede verse influenciada por la resistencia de la planta a las auxinas. En este trabajo, se investigó el impacto de la resistencia a las auxinas en la actividad PGPR de *Lysinibacillus pinottii* sp. nov. PB211. PB211 produjo un promedio de 32 µg mL^{-1} de ácido indol-3-acético (AIA). Evidencia genética indicó que PB211 utiliza la vía del ácido indolpirúvico para la síntesis de AIA. En cuanto a la respuesta de los modelos de plantas al

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tratamiento con AIA, los modelos de eudicotiledóneas (pepino y frijol) mostraron mayor sensibilidad al AIA en comparación con los modelos de monocotiledóneas (maíz y brachiaria). Las monocotiledóneas requirieron concentraciones más altas de AIA para provocar cambios fenotípicos en la arquitectura de la raíz. Se encontró que la resistencia/sensibilidad de las plantas a las auxinas exógenas modula la actividad PGPR de PB211. La inoculación con PB211 en diferentes concentraciones resultó en efectos diferenciados en los modelos de plantas. Las eudicotiledóneas mostraron una actividad PGPR significativa desde concentraciones bajas de inóculo, mientras que las monocotiledóneas requirieron concentraciones más altas de inóculo para exhibir un efecto similar y consistente. El efecto de PB211 también fue evaluado en plantas silvestres de *Arabidopsis thaliana* (col-0 "sensible a auxinas") y mutantes (aux1-7/axr4-2 "resistentes a auxinas"). PB211 tuvo un efecto en forma de "campana" en la respuesta de las plantas silvestres, una respuesta típica de la actividad de auxinas, por lo que el efecto PGPR disminuyó en la concentración más alta de inóculo. Por el contrario, las plantas mutantes exhibieron una mayor actividad PGPR con concentraciones más altas de inóculo, compensando su fenotipo deficiente en auxinas. Estos hallazgos sugieren que la resistencia/sensibilidad de las plantas a las auxinas exógenas influye en la actividad de los PGPR productores de auxinas. Estas relaciones podrían facilitar el desarrollo y la aplicación de inoculantes biológicos más efectivos para la agricultura.

Palabras clave adicionales: monocotiledóneas; eudicotiledóneas; ácido indol-3-acético; promoción del crecimiento de plantas; biofertilizantes; bioestimulantes.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are the most promising microbial group to develop agro-biotechnological products (Kumar *et al*., 2021). However, the lack of knowledge of the soil-plant-bacteria relationship and the difficulty to design efficient formulations affect the reproducibility of PGPR uses (Tabassum *et al*., 2017; Kaminsky *et al*., 2019). Thus, the biofertilizer industry has had a slow advance (Bashan *et al*., 2014).

Auxin synthesis is an important PGPR mechanism, especially indole-3-acetic acid (Spaepen *et al*., 2014). Auxins are plant hormones that allow them to respond to environmental and endogenous stimuli through signaling mechanisms (Chapman and Estelle, 2009; Vanneste and Friml, 2009; Calderon-Villalobos *et al*., 2010; Lavy and Estelle, 2016). Therefore, they regulate

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cell division and elongation, cell differentiation, and shoot and root development (Moller and Weijers, 2009; Perrot-Rechenmann, 2010; Vernoux *et al*., 2010; Overvoorde *et al*., 2010); apparently, bacteria and plants developed this trait through convergent evolution (Patten *et al*., 2013; Yue *et al*., 2014; Blázquez *et al*., 2020).

Although auxin-bacterial production is a PGPR mechanism (Spaepen *et al*., 2014), it can be also a deleterious mechanism (from deleterious rhizobacteria - DRB) under several ecological conditions (Nehl *et al*., 1997; Kremer, 2007; Phukan *et al*., 2021), and it is also a virulence factor of some phytopathogen bacteria (Kunkel and Harper, 2018). Concentration is the key factor to define the role of auxin-bacterial production.

The excess of IAA is converted to 1-aminocyclopropane-1-carboxylic acid (ACC), and this, in turn, is converted into ethylene — the plant growth control hormone (Růzicka *et al*., 2007). Additionally, recent evidence suggests that the auxin excess reduces the content of free TRANSPORT INHIBITOR RESPONSE-1 (TIR1) in the nucleus, and it increases the ubiquitination of TIR/AFB-Aux/IAA co-receptor complex; consequently, the auxin response factors (ARF) are downregulated (Fendrych *et al*., 2018). Both mechanisms delay plant growth. However, the plant response to exogenous auxins varies between plant models and phenological stages (McSteen, 2010; Cao *et al*., 2020).

Therefore, if a bacterial strain utilizes IAA production as a mechanism for plant growth-promoting rhizobacteria (PGPR), the plant's resistance to auxins could potentially modulate its effectiveness. This postulate was recently proposed in a review published by our research group (Pantoja-Guerra *et al*., 2023b). In this study, we investigate the impact of this effect on the PGPR activity of *Lysinibacillus pinottii* sp. nov. (PB211). These considerations bear significant implications for the design, formulation, and utilization of biological inoculants in agriculture.

MATERIALS AND METHODS

Bacterial strain

The PB211 strain was recently identified as *Lysinibacillus pinottii* sp. nov. (Dunlap *et al*., 2024) and was supplied by Pathway-Biologic® (now Mosaic, Inc.). This strain was isolated for bioprospecting purposes. The PGPR activity of PB211 associated with IAA production was previously evidenced (Pantoja-Guerra *et al*., 2023a). The strain was kept frozen at -80°C and the reactivation was done on Trypticase Soy Agar (TSA), incubated at 28°C.

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IAA bacterial production

PB211 was grown on TSA and incubated at 28°C for 48 h. A single colony was then transferred to 100 mL flasks containing 20 mL of Trypticase Soy Broth (TSB) supplemented with 500 µg mL^{-1} of tryptophan and shaken at room temperature for 48 h. After incubation, the flask contents were centrifuged at 3,250 rpm for 15 min. Then, 200 µL of the supernatant were mixed with 800 µL of Salkowski reagent. The mixture was left to stand for 20 min until a pink color developed. To determine the IAA concentration in the bacterial broth, absorbance values were interpolated using a standard curve of known IAA concentrations. Measurements were taken five times.

Furthermore, the complete genome of PB211 was sequenced, and the obtained data were used to identify genes associated with IAA production. All procedures for the functional annotation of the genome, as well as the detection and identification of the reported genes and enzymes, were previously described for PB211 (Pantoja-Guerra *et al*., 2023a).

Effect of IAA on plant models with differential sensitivity to auxins

The natural resistance of monocots to auxin activity was used to evaluate the IAA effect on different regimes of auxin sensitivity in plants (McSteen, 2010; Balzan *et al*., 2014). A first screening including several plant models allowed us to determine that monocot plants were more resistant to IAA activity than eudicot plants. That is, higher concentrations of IAA were required to observe plant phenotypic changes. In this way, a confirmatory experiment was performed to obtain evidence of differential response of monocot and dicot plants to auxins.

Corn (*Zea mays* - 'ICA109'), brachiaria (*Brachiaria decumbens* cv. Basilisk), cucumber (*Cucumis sativu*s - 'Pointsett76'), and bean (*Phaseolus vulgaris* - 'cargamanto mocho') seeds were carefully selected and disinfected by immersing them in 70% ethanol for 5 min. Afterward, they were rinsed five times with sterile distilled water and then dried on sterile absorbent paper. Next, the seeds were placed in agar-agar plates and incubated in darkness at 28°C for 3 d until a radicle of 1-2 cm developed. Then, three seedlings were transferred to an agar dish to constitute an experiment unit. The evaluated treatments for corn and brachiaria (monocots) were 0 (control), 1.0, 2.5, 5.0, 7.5, and 10.0 mg L^{-1} of IAA, while for cucumber and beans (eudicots), the treatments were 0 (control), 0.010, 0.050, 0.175, 0.250, 0.500, 0.750, and 1.000 mg L^{-1} of IAA. A preliminary assessment indicated that monocot seedlings do not exhibit a significant response to

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low IAA concentrations. To each seedling of the treatments, 500 µL were added to the respective IAA solution; in control treatments, 500 µL of sterile distilled water were added. Each treatment was evaluated with three repetitions and all experiments were replicated two times. The agar dishes with the seedlings were incubated at room temperature and under room light conditions. Monocot experiments were harvested 5 d after the treatment addition, and eudicots were harvested 3 d later.

After the incubation period, thinning was done and the measurements of the plant on each agar dish were performed. To collect the root architecture data, an image digital analysis was performed with the Smart Root package (version 4.1). The photography conditions were optimized according to our laboratory facilities.

Effect of PB211 on plant models with differential sensitivity to auxins

To determine whether monocot and dicot plants respond differently to PB211 depending on inoculum concentration, greenhouse experiments were conducted. Corn, brachiaria, cucumber, and bean seeds were selected, disinfected, washed, dried, and pre-germinated following the previously described procedure.

An initial screening helped optimize the most relevant PB211 concentrations for each plant model. Fourteen seedlings of each species were immersed in their respective bacterial spore suspension for 1 h. The treatments consisted of sterile distilled water (control), 10^4, and 10^8 colony-forming units (cfu). The seedlings were then transplanted into polyvinyl chloride (PVC) pots measuring 25 cm in length and 8 cm in diameter. To eliminate soil auxin activity, river sand was used as the growth substrate. A 50% Hoagland nutrient solution was applied to the sand during irrigation, maintaining its maximum moisture retention capacity throughout the experiment.

Each pot contained two seedlings, and 1 mL of the respective bacterial suspension was inoculated at the root of each plant. The experiment followed a completely randomized design with seven replicates per treatment. The entire experiment was conducted twice, and data were analyzed using time blocking.

Monocot plants were harvested 2 weeks after planting, whereas dicot plants were harvested after three weeks. Before data collection, thinning was performed to retain only one plant per pot. For monocots, the measured response variables included shoot and root dry weight, total root length, number of lateral roots, and number of primary roots. In dicots, the evaluated parameters

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were shoot and root dry weight, total root length, lateral root length, and the number of lateral roots. Root architecture parameters were assessed using digital image analysis with the SmartRoot package (Lobet *et al*., 2011; Lobet *et al*., 2015), with photography conditions optimized according to the greenhouse setup.

To determine if the inoculum concentration affects the IAA concentration in the growth bacterial medium, a parallel experiment with PB211 was performed. The bacterial production of IAA was evaluated as described above, but in this case, the agitation period was only 24 h. A completely randomized experiment with five repetitions was performed. The evaluated treatments were control (only culture medium), 10^4, 10^6, and 10^8 cfu of PB211.

PB211 auxin-like activity. Effect on corn and cucumber root hairs

To obtain another evidence line of the role of the auxin-bacterial production in the PB211 PGPR activity on agronomic models, an *in vitro* experiment was performed. The inoculum concentrations detected in the previous trial as significant for the growth promotion of monocots and eudicots were evaluated on the root hair development of corn and cucumber, respectively. This effect was compared with the IAA concentration previously established as significant for each plant model. In this way, the treatments evaluated in corn were control (sterile distilled water), IAA (2.5 mg L^{-1}), and PB211 (10^8 cfu). The treatments evaluated in cucumber were control (sterile distilled water), IAA (0.05 mg L^{-1}), and PB211 (10^4 cfu).

The seeds were pre-germinated in agar-agar, once seedling radicules were 1 cm long they were transferred individually to agar plates; thus, each one constitutes an experiment unity. 1 mL of each suspension treatment was added to seedling radicules. A completely random experiment was performed with ten repetitions. The seedlings were kept under room conditions for one week. After the incubation period, 1 cm-segments of the main root were cut at 2 cm from the root base of each plant. The root hairs were observed with an optic microscope — 4X objective — using a millimetric length pattern. The variables evaluated were average length of hairs and hair density (number of hairs per root mm). These were estimated by image digital analysis using the ImageJ/Fiji package (2.16.0).

Effect of PB211 inoculum concentration on plant growth of *Arabidopsis thaliana* col 0 and the mutant aux1-7/axr4-2

The aux1-7/axr4-2 mutants exhibit defects in the coding of a protein responsible for facilitating the transport of IAA from the leaves to the root tips, as well as in the protein's polar

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localization. As a result, these mutants have a lower IAA concentration in the roots, leading to a dwarf phenotype (Puga-Freitas *et al*., 2012). Additionally, this mutant shows increased resistance to IAA, meaning that higher concentrations of IAA are required to induce noticeable phenotypic changes in the plant. If the PGPR activity of PB211 is influenced by the plant's resistance or sensitivity to auxins, growth patterns will differ between wild-type and mutant plants depending on the inoculum concentration.

The seeds were placed in Petri dishes containing MS medium using a micropipette tip, following the method described by Rivero *et al*. (2014). They were kept at 4°C for 48 h for stratification and then pre-germinated in a Percival® E36-HO growth chamber under controlled conditions (25°C, 12-h photoperiod, and light intensity of 1,700 µmol m^{-2} s^{-1}) for 2 weeks.

After pre-germination three seedlings were transferred to Petri dishes with MS medium, forming an experimental unit. A completely randomized experiment was conducted with four replicates for both wild-type and mutant *A. thaliana* plants. The tested treatments included a control and PB211 inoculum concentrations of 10^4, 10^6, and 10^8 cfu. Bacterial suspensions were inoculated 3.4 cm away from the plants. The Petri dishes were then incubated in growth chambers under the previously described conditions for two weeks.

As response variables, plant fresh weight and root architecture parameters were measured using the SmartRoot package (version 4.1) (Lobet *et al*., 2011; Lobet *et al*., 2015).

Statistical analysis

All data were examined using linear models through an analysis of variance (ANOVA). Significant differences between treatments and the control were identified using the Dunnett test, with P values reported in all cases. When necessary, the Tukey test (alpha = 0.05) was applied to compare differences among all treatments.

RESULTS AND DUSCUSSION

IAA bacterial production by PB211

The average IAA production by PB211 was 32 µg mL^{-1} (Fig. 1A). An aliquot of the control was taken as a blank. The IAA concentration observed in Fig. 1A corresponds to the net IAA production — it is not necessary to illustrate the control treatment. This result was similar to preliminary tests performed by HPLC (High-Performance Liquid Chromatography) (data not

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shown). Based on genetic evidence, we inferred that PB211 uses a tryptophane-dependent pathway to synthesize IAA: the indole pyruvic acid pathway (Fig. 1B).

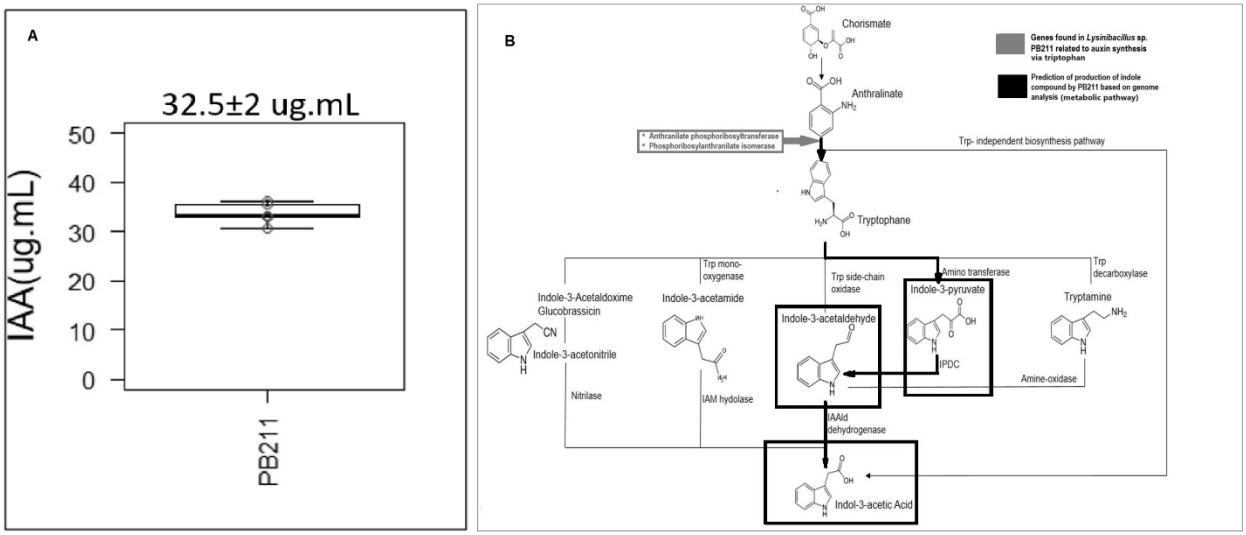

Figure 1. IAA production by *Lysinibacillus pinottii* sp. nov. PB211. A, box-plot chart plus strip-chart showing the amount of net IAA in liquid medium supernatant. The value displayed is the average of five repetitions. B, genetic analysis of IAA production in PB211. Gray labels indicate genes—either tryptophan-dependent or independent—identified in PB211 and associated with auxin biosynthesis. Black labels represent the predicted IAA synthesis pathway in PB211, adapted from Pantoja-Guerra *et al*., (2023a).

The ability of *Lysinibacillus* sp. genus to produce IAA has been previously documented (Naureen *et al*., 2017; Castellano-Hinojosa *et al*., 2018; Jinal *et al*., 2021; Pantoja-Guerra *et al*., 2023a). Recently, we provided evidence supporting the causal relationship between IAA production and the PGPR activity of *Lysinibacillus spp*. (Pantoja-Guerra *et al*., 2023a). The IAA production reported in this study aligns with previous findings on the subject (Mohite, 2013); however, the *Lysinibacillus* genus generally exhibits higher IAA production than other members of the Bacillaceae family (Kim and Song, 2012; Naureen *et al*., 2017; Pal and Sengupta, 2019).

Genomic analysis suggests that the predicted metabolic pathway for IAA biosynthesis in PB211 follows the indole-3-pyruvic acid (IPyA) pathway. This pathway is widely distributed among Firmicutes and is considered the primary route for IAA production in Bacilli (Shao *et al*., 2015; Zhang *et al*., 2019; Alviar *et al*., 2021). A detailed list of auxin-related genes identified in PB211, along with a predicted pathway for indole compound production based on genome analysis, is available in Online Resource.

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Effect of IAA on plant models with differential sensitivity to auxins

The eudicot models showed higher sensitivity to IAA than the monocot models. Higher doses of IAA were required to observe phenotypic changes in root architecture in monocot plants. A first screening allowed us to determine that corn seedlings do not show a significant response to low doses of IAA (Fig. 2A). Corn and brachiaria seedlings responded to the IAA treatment in a concentration range from 0-10 mg L^{-1} (Fig. 2 D and E), while bean and cucumber plants showed phenotypic changes in the root system with an IAA concentration range from 0-1 mg L^{-1} (Fig. 2 B and C).

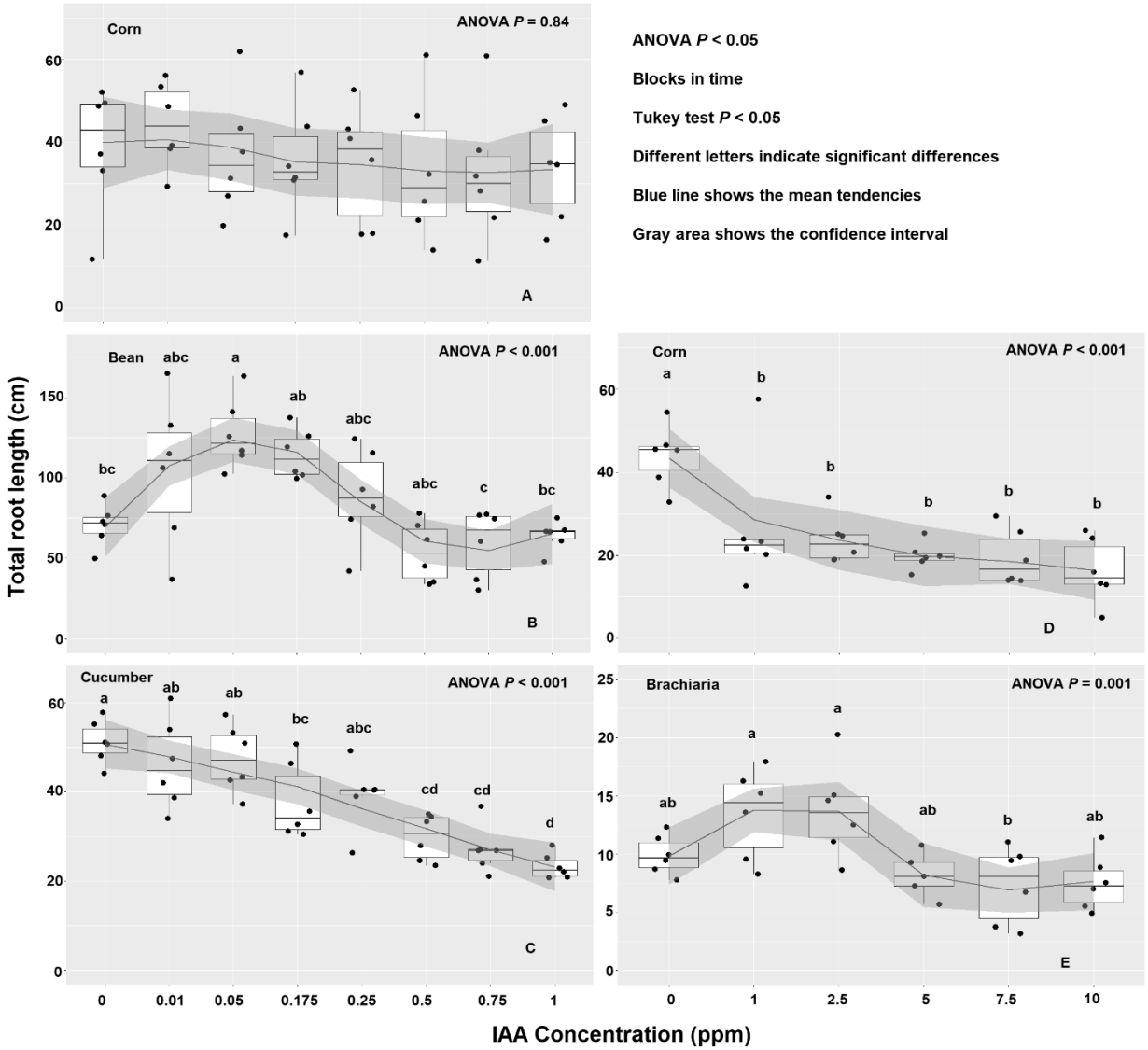

Figure 2. Dose-dependent effect of exogenous application of IAA on the total root length of monocots (A, corn 0-1 mg L^{-1} of IAA; D, corn 0-10 mg L^{-1} of IAA; E, brachiaria) and eudicot (B, bean; C, cucumber) models.

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These results agree with the findings of Haga and Lino (1998), who compared the sensitivity of corn (monocot) and pea (eudicot) tissues to IAA through an *in vitro* assay. Corn coleoptile elongation could be observed only at an IAA concentration one logarithmic order higher than the concentration required to obtain the same effect in pea internodes. Usually, monocot plants are more resistant to exogenous auxin action (Aloni and Plotkin, 1985; Scarpella and Meijer, 2004; McSteen, 2010). Therefore, higher IAA concentrations are required to observe changes in the phenotype of monocot plants than in eudicot models.

This phenomenon has been explained in several forms. The fibrous composition of adventitious roots makes monocot plants less sensitive to exogenous auxin because it allows hormone degradation before it starts its activity (McSteen, 2010). Masuda (1980) compared oat coleoptiles cell wall composition and azuki epicotyls treated with IAA. During the elongation, the dicots' cell wall hemicellulose is constituted by xyloglucans and galactans as opposed to monocots, which have a higher content of cellulose microfibrils. This could be associated with resistance to the exogenous auxin effect.

On the other hand, two cotyledons facilitate auxin transport during the embryonic stage and enable fast tissue differentiation and early growth. In this way, dicot plants are more sensitive to the action of exogenous auxins (McSteen, 2010). Most plant endogenous auxins are synthesized in the shoot apical meristem (SAM), and the growth of leaves from the SAM is repressed by KNOX protein. The latter has an expression area proportional to the distribution of SAM and it is downregulated by synthesis or exogenous addition of auxins. The SAM has a well-delimited location at the base of leaves in eudicot plants. Thus, the expression of the KNOX area is substantially smaller than in monocot plants, where the base of leaves surrounds the pseudostem plant circularly and the SAM is spread out (Conklin *et al*., 2019). Therefore, the auxin stimulus in monocot plants must be greater for adequate plant growth.

The growth regulation of monocot and eudicot leaves differs spatially and temporally. The SAM is continued by an active division zone, which allows leaf growth. This growth is slowed down by the cell cycle arrest front, which appears faster in dicot plants, thus starting the spatial regulation by cell expansion. Monocot leaves maintain the division zone longer because they are dependent on temporal regulation (Nelissen *et al*., 2016). The active division zone functionality depends on auxin synthesis, flux and signaling; therefore, this characteristic can be associated causally with the response of monocot models to higher IAA concentrations.

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The auxin endogenous flux (polar transport and homeostasis) is more efficient in eudicot than in monocot plants; they require lower auxin concentrations to express their activity (Balzan *et al*., 2014). This efficiency is explained by two points: 1) monocot plants have a greater number of auxin transport protein families, which increases the subfunctionalization probability (Balzan *et al*., 2014); 2) the eudicot leaves develop a reticulated interveinal system consisting of a middle vein, lateral veins, and minor veins, which are configured in a loop shape that feeds itself (McSteen 2010; Conklin *et al*., 2019). Therefore, epidermally located PIN proteins converge, thus improving the efficiency of polar auxin transport. Eventually, it can increase their sensitivity to exogenous auxins.

This does not occur in monocot models, which develop parallel interveinal systems (Conklin *et al*., 2019); however, although monocots are more resistant to auxin than eudicot plants (Scarpella and Meijer, 2004; McSteen, 2010), the plant reticulated vascularization has high plasticity. Some plant models do not fit into this generalization (Scarpella and Meijer 2004; Conklin *et al*., 2019; Chen *et al*., 2018). We evaluated other models in preliminary screening, e.g., radish responded similarly to eudicots, but the tomato seedlings did not fit this pattern (data not shown).

Differential effects of *Lysinibacillus pinottii* sp. nov. PB211 PGPR activity associated with the plant models' auxin resistance

The PGPR activity by PB211 was expressed by cucumber and bean (eudicots) with an inoculum concentration of 10^4, opposite to corn and brachiaria (monocots) where PGPR activity was observed consistently at 10^8 (Tab. 1).

Previously, we discussed the differential effect of the IAA concentration on mono and eudicot plant models. PB211 auxin production has been associated directly with its PGPR effect (Pantoja-Guerra *et al*., 2023a). Evidence that the inoculum concentration of auxin-producing bacteria has a dose-effect on plants similar to the exogenous auxin application has been published (Malik and Sindhu 2011; Suarez *et al*., 2014; Mangmang *et al*., 2015; Kudoyarova *et al*., 2017; Kudoyarova *et al*., 2019). The different patterns caused by the inoculation of PB211 on the plants tested here suggest that the level of plant resistance/sensitivity to exogenous auxins modulates the PGPR effect.

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Table 1. Differential response of mono and eudicot plant models to the inoculation of *Lysinibacillus pinottii* sp. nov. PB211. A, statistical significance of bacterial inoculation on plant morphological parameters on monocots and eudicots (mean comparisons by Dunnett test). B, plant response to a lower and a higher inoculum concentration of PB211 on eudicots and monocots, respectively. The images of all the experiments can be seen in online resource.

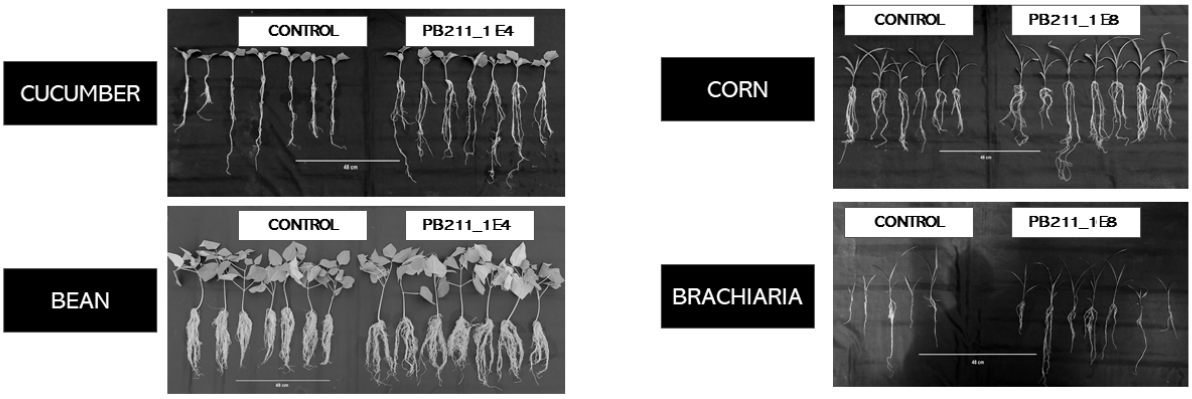
A

MONOCOTS	PB211 - Inoculum concentration	Shoot dry weight	Root dry weight	Total length root	Number of lateral roots	Number of primary roots
Corn	Control					
	1:E4					
	1E8	*	*	***	*	***
Brachiaria	Control					
	1:E4		*			
	1E8	*	**	**	**	**

EUDICOTS	PB211 - Inoculum concentration	Shoot dry weight	Root dry weight	Lateral length root	Total length root	Number of lateral roots
Cucumber	Control					
	1:E4	**	**	***	***	***
	1E8	*	*			
Bean	Control					
	1E4		*	***	***	***
	1E8	*	**	*	*	***

P value	< 0.05	< 0.01	< 0.001
	*	**	***

B



Likewise, the results presented in online resource indicate that the inoculum concentration directly affects the IAA bacterial production in liquid culture. This result agrees with Bharucha *et al*. (2013), Kudoyarova *et al*. (2017), and Bunsangiam *et al*. (2021) who, from an empirical approach, concluded that bacterial IAA production under batch conditions is directly proportional to the bacterial concentration in the liquid culture.

Moreover, auxin bacterial production directly or indirectly affects other PGPR mechanisms such as ACC deaminase production (Glick *et al*., 1997; Khan *et al*., 2016; Duca *et al*., 2018), and phosphorus bacterial solubilization (Ramírez and Kloepper 2010; Kudoyarova *et al*., 2017), among others (Kudoyarova *et al*., 2019). Therefore, additionally, to the auxin plant resistance, the inoculum concentration of IAA-producing bacteria may be linked to other PGPR mechanisms not evaluated in this work.

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PB211 auxin-like activity. Effect on corn and cucumber root hairs

The PB211 (10^8 cfu) inoculation in corn seedlings had an effect similar to the treatment with 2.5 mg L^{-1} IAA on root hairs (Fig. 3). Likewise, the PB211 (10^4 cfu) inoculation in cucumber caused a root hair development similar to the treatment with IAA 0.25 mg·L^{-1} (Fig. 4).

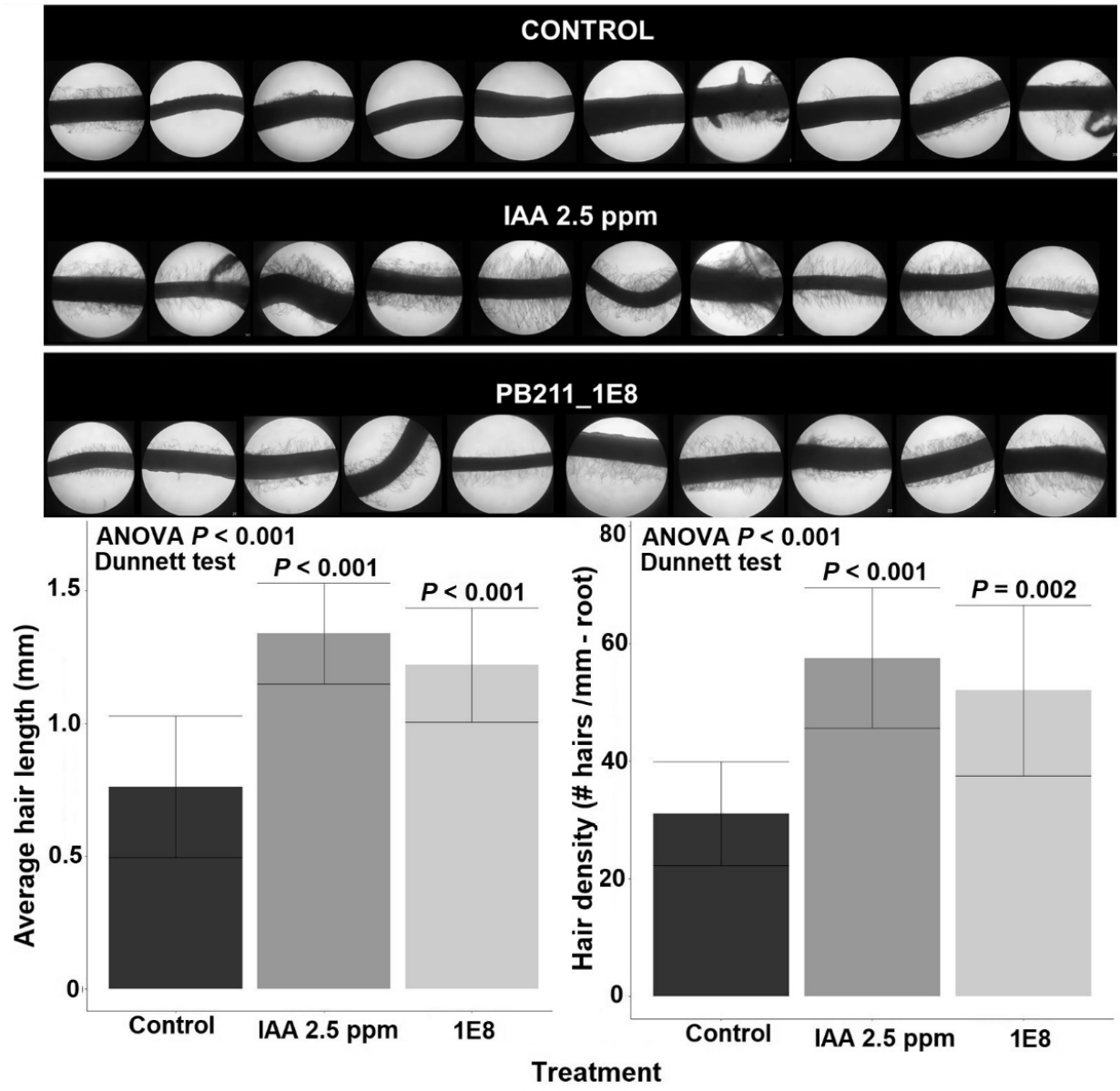

Figure 3. Response of corn seedlings root hairs to PB211_1E8. The effect was compared with the IAA treatment (2.5 mg L^{-1}). A complete randomized trial with ten replicates was performed. Significant differences were calculated with Dunnett test. The *P* values of the control means comparison are shown on each treatment. The measured variables were average hair length and hair density (number of hairs per mm of root). Field of view diameter: 4,000 µm.

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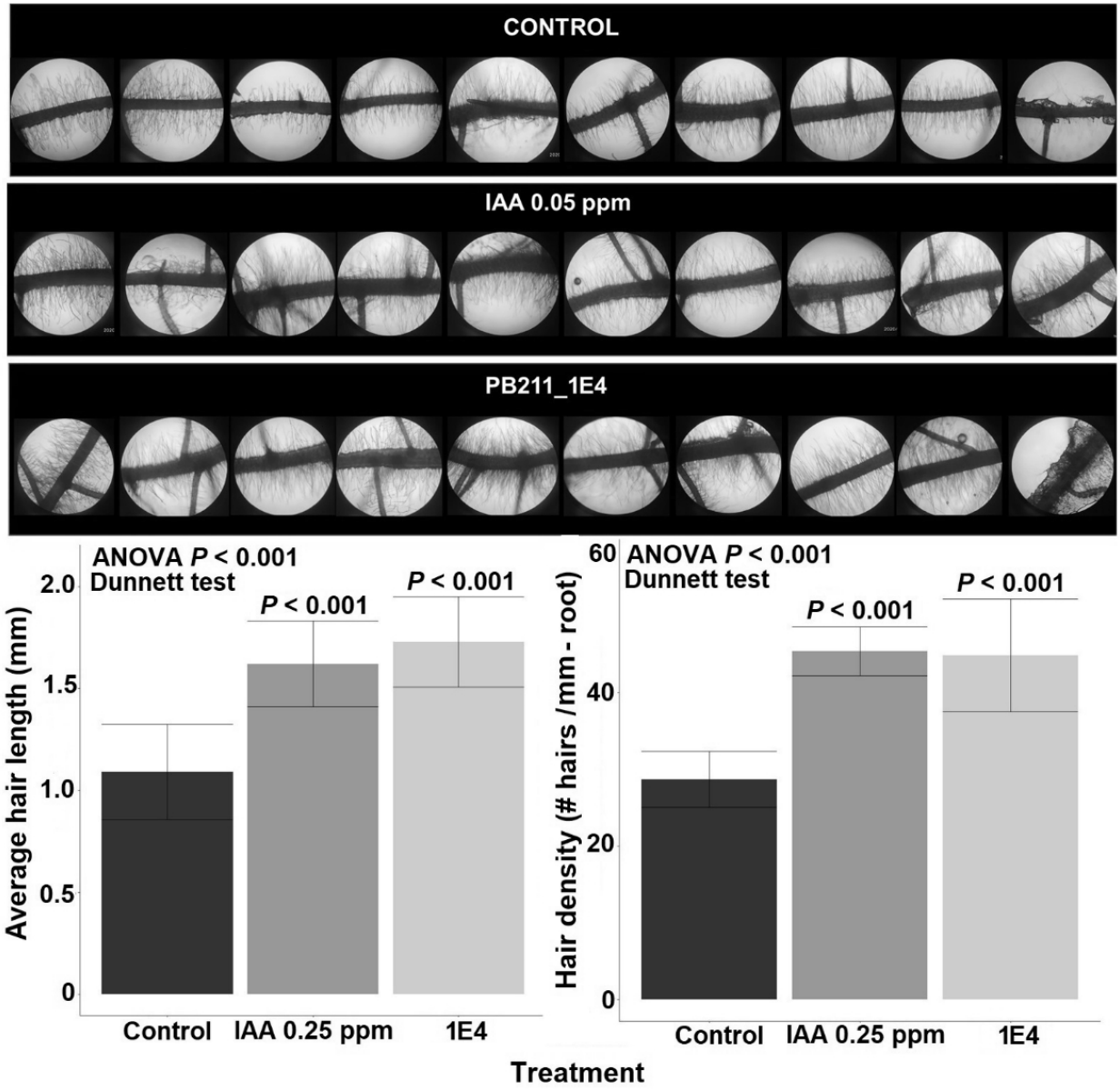

Figure 4. Response of cucumber seedlings root hairs to PB211_1E4. The effect was compared with the IAA treatment (0.05 mg L^{-1}). A complete randomized trial with ten replicates was performed. Significant differences were calculated with Dunnett test. *P* values of the control means comparison are shown on each treatment. The measured variables were average hair length and hair density (number of hairs per mm of root). Field of view diameter: 4000 µm.

In both plant models (corn and cucumber), each inoculum dose of PB211 exhibited a comparable effect on root hair production to the dose of IAA with which it was compared, thereby providing further evidence of PB211's auxin activity as a PGPR mechanism.

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Root hairs are root surface-modified cells, they play a key role in nutrient absorption and recognition of the environmental factors. Thus, the length and number of root hairs are linked to auxin signaling. Some genetic elements responsible for the expression of root hairs depend on auxin signaling and respond to exogenous auxin addition, e.g., some auxin response factors (ARF) (Lee and Cho, 2013; Vissenberg *et al*., 2020). Therefore, the change in the organization pattern of root hairs is a suitable trait to evaluate the auxin effect. This result suggests the auxin activity of PB211 on these agronomic models.

Effects of endogenous auxin signaling on the *Arabidopsis thaliana* response to PB211 inoculation

The PGPR activity of PB211 on *A. thaliana* col 0 (wild) after the inoculation of 10^4 and 10^6 cfu was observed. This effect was lost with the 10^8 cfu treatment, which did not show differences with the control. The inoculum concentration generated a parabolic-type growth pattern on wild plants (Fig. 5). The 10^8 treatment caused a similar effect on 0.25 mg L^{-1} IAA; however, contrary to PB211_10^8 cfu, the IAA treatment had a significant effect on root architecture variables (Fig. 5).

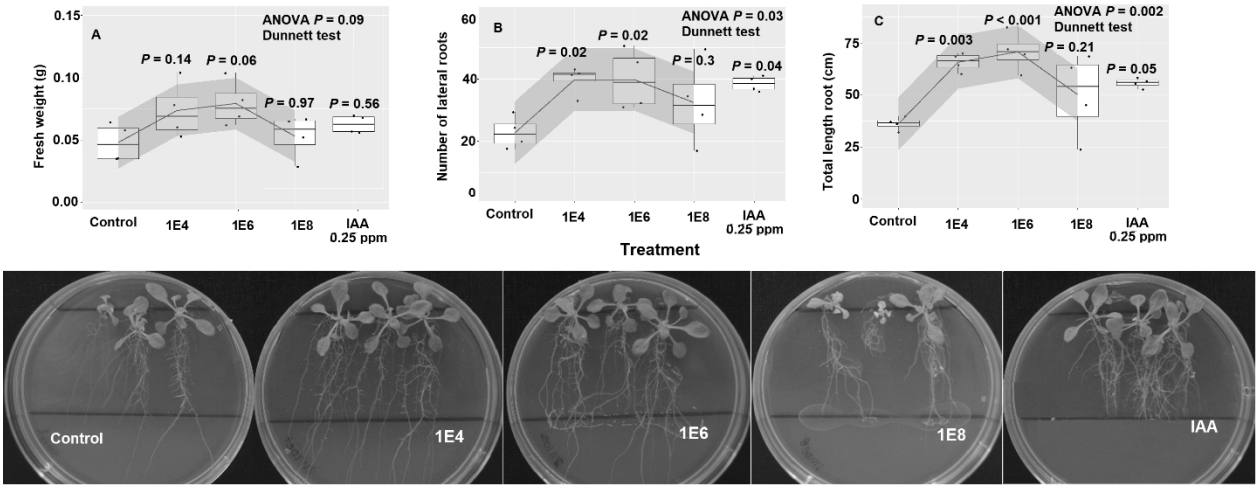

Figure 5. Dose-dependent response of *A. thaliana* col 0 (wild) to the inoculation of PB211. A, fresh weight. B, number of lateral roots. C, total root length. The Dunnett test was used to perform mean comparisons. The line shows the mean tendencies for each treatment, and the gray area shows the confidence interval.

The PB211 inoculum concentration generated an exponential-type growth pattern in *A. thaliana* aux1-7/axr4.2 (mutant). The PGPR activity of PB211 increased proportionally to the inoculum concentration (Fig. 6). Only the 0.25 mg L^{-1} IAA and the 10^8 treatment had a

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significant effect on the fresh weight and root architecture of mutant plants (Fig. 6). The *Lysinibacillus pinottii* sp. nov. PB211 PGPR activity on *A. thaliana*, as well as the role of IAA bacterial production on said activity, were previously demonstrated (Pantoja-Guerra *et al*., 2023a). However, the inoculum concentration effect on these plant models was unknown.

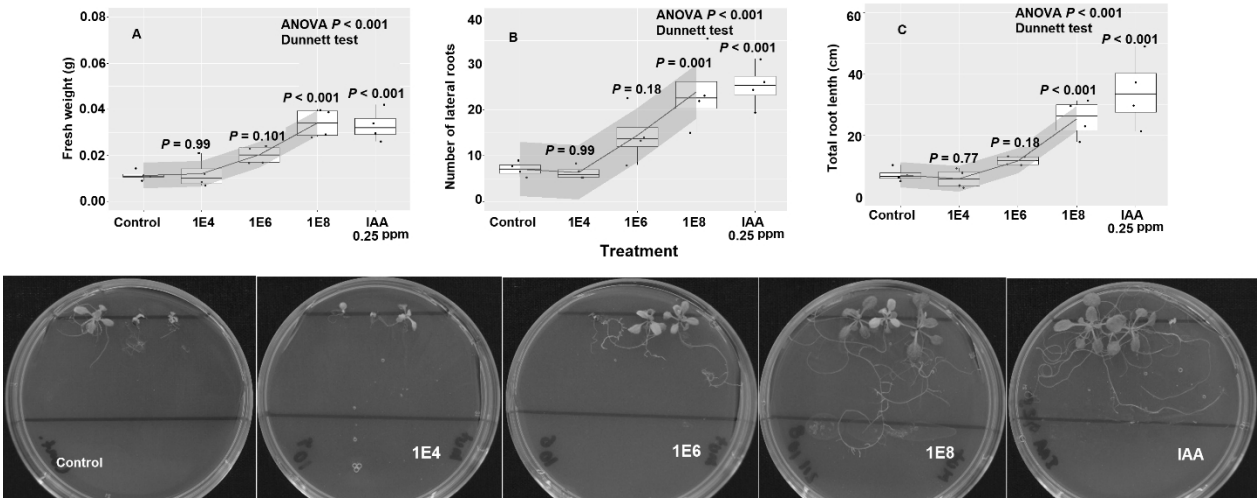

Figure 6. Dose-dependent response of *A. thaliana* aux1-7/axr4-2 (mutant) to the inoculation of PB211. A, fresh weight. B, number of lateral roots. C, total root length. The Dunnett test was used to perform mean comparisons. The line shows the mean tendencies for each treatment, and the gray area shows the confidence interval.

Arabidopsis thaliana col 0 is a eudicot plant without defects in auxin signaling and transport. Therefore, it is more sensitive to exogenous auxin activity than its mutant version aux1-7/axr4-2. The PGPR effect of PB211 in wild plants was observed up to 1E6 and it disappeared at 1E8; thus, the IAA effect was emulated. This upward growth up to a specific concentration and then downward (bell type) is typical of auxin activity on plants (Woodward, 2005). This effect has also been described for the inoculation of auxin-producing bacteria (Suarez, 2014; Kudoyarova *et al*., 2017). These results and reasonings cited here allow us to causally associate the A. thaliana col 0 response to PB211 with the auxin sensitivity of the plant model.

Opposite to wild plants, in the mutant model of A. thaliana aux1-7/axr4-2, the auxin transport from the leave meristems to the roots is atrophied. Therefore, an auxin-deficient dwarf phenotype plant with a poor root system is generated (Hobbie and Estelle, 1995; Swarup *et al*., 2004; Dharmasiri *et al*., 2006; Puga-Freitas *et al*., 2012; Blouin, 2018). This phenotype is more resistant to exogenous auxin activity than wild plants due to the lack of IAA in the root system (Hobbie and Estelle, 1995). Thus, a high inoculum concentration supplies the auxin amount required to

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express phenotype changes in mutant plants, and the plant model response to the inoculum is a result of its exogenous auxin resistance level.

These results agree with the table 1. Models more resistant to auxins (monocots) showed a response similarly to that of *Arabidopsis* mutant plants to inoculum concentration. Conversely, the more sensitive models (eudicots) showed a growth-promoting effect at 1E4, similar to the wild *Arabidopsis* plants. This evidence suggests that the resistance/sensitivity of plants to exogenous auxins could explain, at least partially, this pattern results. However, other environmental factors must be considered.

CONCLUSION

The PGPR activity of *Lysinibacillus pinottii* sp. nov. PB211 varied depending on the plant's sensitivity or resistance to exogenous auxins. In cucumber and bean plants, PB211 inoculation stimulated growth at a concentration of 1E4, whereas in corn and brachiaria, this effect was consistently observed at 10E8. Similarly, Arabidopsis mutant plants (*aux1-7/axr4-2*), which exhibit greater resistance to exogenous auxins than wild-type (*Col-0*), required higher inoculum doses to partially reverse the mutant phenotype. These findings suggest that PB211's PGPR activity is modulated by the plant's auxin sensitivity.

This study provides evidence of the auxin-resistance effect in certain plant models when exposed to the auxin-producing strain *Lysinibacillus pinottii* sp. nov. PB211. The results highlight that PGPR effectiveness depends on the plant’s ability to respond to auxins, which has significant implications for the formulation and application of biological inoculants in agriculture. Understanding these interactions enables a more targeted and efficient use of microbial inoculants, promoting sustainable agricultural practices and improving crop productivity.

Conflict of interests: The authors declare that they have no competing interests.

Author’s contributions: M.P.-G., carried out the in-planta experiments and drafted the manuscript. C.A.R., reviewed the manuscript. Both authors approved the final version of the document.

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Open source research data repository: **Online resources RCCH_Pantoja_Guerra (2025).pdf**

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