Pantoja-Guerra *et al.*
Chem. Biol. Technol. Agric. (2023) 10:6
https://doi.org/10.1186/s40538-022-00370-8

Chemical and Biological Technologies in Agriculture

REVIEW

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Total auxin level in the soil–plant system as a modulating factor for the effectiveness of PGPR inocula: a review

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Abstract

Biofertilizers are an alternative to face the sustainability problem that chemical fertilizers represent in agriculture. Among them, plant growth-promoting rhizobacteria (PGPR) is a microbial group with high potential, but lack of reproducible results from their application is a bottleneck for its use in agricultural production. Here we highlight a factor that could partially explain this inconsistency: the total auxin level in the soil–plant system. Auxin production is recognized as a main mechanism for plant growth promotion by PGPR; however, the final effect of auxins depends on a fine balance of its content, and this will be a result of all the sources of auxin compounds in the system. In addition to the auxins produced by inoculated bacteria, the plant itself produces its own hormones as part of complex physiological processes, varying in amount and sensitivity. Also, soil organic matter displays like auxin activity, causing plant responses just like those produced by added auxins. Therefore, the inoculation of an auxin-producing PGPR on plants might cause a wide variety of responses, ranging from effective growth promotion to growth restriction, depending on the total auxin content in root tissue. We think this must be considered for the practical use of bacterial biofertilizers, in order to have better and more consistent results of inoculation.

Keywords Biofertilizers, PGPR, Auxin-like effect

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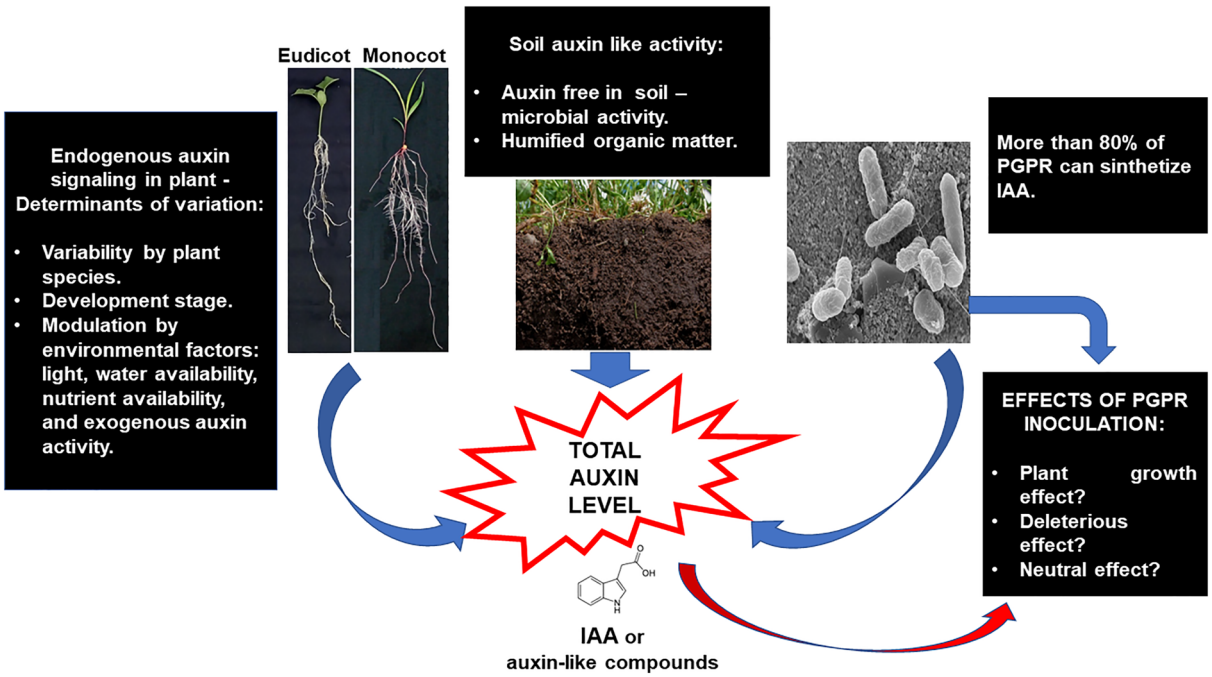
Graphical Abstract



Introduction

Biofertilizers are *"biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote growth by several mechanisms"* [1]; these constitute a biotechnological, sustainable, and environmentally convenient alternative to increase agricultural productivity in the face of the challenges posed by climate change and land degradation [2].

The potential of biofertilizers in sustainable agricultural development has allowed an exponential increase in the number of scientific publications on this subject since the last decade of the past century. In addition, the global biofertilizer market was valued at USD 2.6 billion in 2021, and it may reach USD 4.5 billion by 2027, growing at a CAGR (compound annual growth rate) of 12% [3]. This indicates an actual tendency towards the use of such products in agriculture.

Plant growth-promoting rhizobacteria (PGPR) are the microbial group that offers the greatest potential for biofertilizer formulation because bacterial cells grow faster under laboratory conditions, and it is easier to perform scale processes [4]. Furthermore, there is robust evidence on the mechanisms involved in plant growth-promoting (PGP) and increases in agricultural production using PGPR have been reported [5].

However, the use of PGPR as an input in biofertilizer production still poses basic topics to be solved, cases reported as successful are not consistent and there is still a lack of results reproducibility [6]; these aspects have hampered further development in the biofertilizer industry [7].

Bacterial indole-3-acetic acid (IAA) production is one of the leading plant growth promotion mechanisms described in PGPR [8–10]. More than 80% of bacteria associated with the rhizosphere can synthesize IAA [11–13]. The production of IAA by rhizosphere bacteria is often selected as a desirable trait in bacterial strains for biological inoculants design [14]. Although IAA bacterial production is a PGP trait, this also is a virulence factor in plant-pathogenic microorganisms [12, 15], and a mechanism in deleterious rhizobacteria [16–18].

Since the 1990s, publications associated with the isolation of IAA-producing bacteria have increased exponentially (Fig. 1). A rapid search in databases, repositories, and scientific search engines provides evidence for the number of publications using the bacterial production of IAA as a selection trait of isolates with PGPR potential (Fig. 1). It is important to highlight noteworthy that according to Scopus, 76% of publications on this topic is distributed among countries with emerging economies

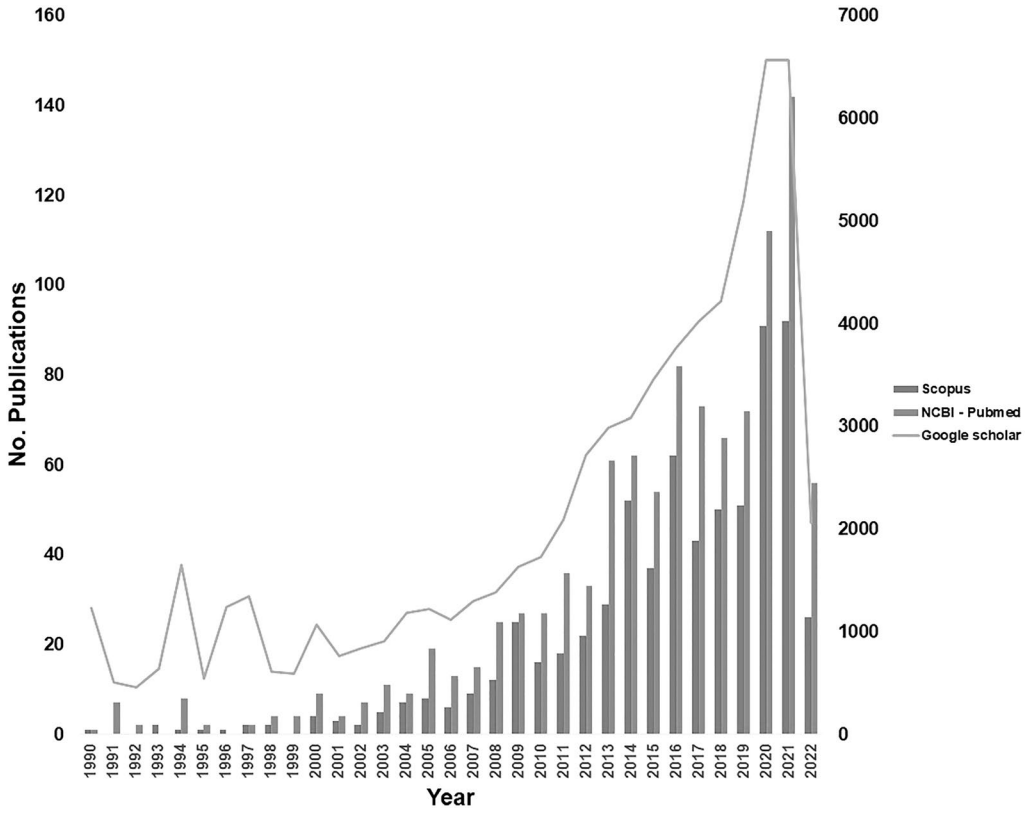


Fig. 1 Bibliometrics for "bacteria""IAA""isolation"

(India, China, Pakistan, Brazil, Thailand, Indonesia, Mexico, Argentina, Malaysia, and Egypt), which could be related to the need to develop agricultural, sustainable, and economically convenient technologies.

In addition to the production of IAA or auxin-like compounds by bacteria, and the endogenous content of plant auxins, the soil also has high auxin-like activity [19]. Early reports refer to the content of water-extractable IAA in the soil labile organic fraction [20– 22]. Another part of the soil organic matter involved in auxin activity is the humified fraction; this phenomenon is called the "auxin-like effect" of humic substances [23–26]. The hormone activity of humified organic matter has given rise to the emergence of products of agrobiotechnological interest formulated based on humic substances [25, 27, 28].

The overexposure of plants to the auxin activity in the rhizosphere, when these are inoculated with IAA-producing bacteria, could have negative effects on plant growth and it could explain part of the inconsistency of the inoculation of PGPR in planta. Much has been written about the central of role auxins for plant growth and its multiple sources, including the plant itself, PGPR, soil native microbes, and soil organic matter. However, a comprehensive review articulating all these components is still lacking.

Here, we do not intend to address technical or theoretical details such as the bacterial IAA synthesis pathways or types of bacterial auxin-like compounds. It is a reasonable and argued opinion on a specific hypothesis: *"total auxin level in the soil–plant system could partially explain the inconsistent results frequently obtained when PGPR are inoculated"*. This hypothesis has practical implications for the research and application of biofertilizers. Some data shown in this review are preliminary results of the authors. Therefore, the complete information is part of future publications that will search for the answer to the questions derived from this research idea.

IAA production in PGPR

In bacteria, IAA production is part of the metabolic transformation mechanisms of tryptophan [29]. The available tryptophan can be used directly by bacteria for the synthesis of proteins [30], and nitrogen supply due to the amine groups released by its catalysis [30–32]. The enzyme Trp-aminotransferase catalyzes the deamination

of tryptophan during the IAA synthesis through the indole pyruvic acid pathway [12, 33]. However, excess tryptophan inhibits bacterial growth [31]. In this way, Patten et al. speculatively proposed that a function of the bacterial production of IAA is the detoxification of excess tryptophan [34]. Furthermore, recent evidence suggests that the bacterial production of IAA is a protective mechanism against oxidative stress [35].

Auxins act by signal transduction mechanisms, so their role as a positive or deleterious factor to plant growth is dependent on concentration. Exogenous auxin excess generates damages in a hormonal loop regulation system, and it can increase the ethylene concentration in roots. So, a decrease in plant growth is observed [36]. In this way, this trait could be plant-deleterious under some environmental conditions [8].

IAA production as a PGPR mechanism

IAA production by rhizobacteria undoubtedly plays a crucial role in the development of plants [6], and there is evidence regarding the PGP effect of IAA-producing bacteria. The effects of wheat seedlings inoculation with the mutant strain *Azospirillum brasilense* SPM7918, a low IAA producer, were estimated. Compared with the wild-type strain Sp6, SpM7918 showed a reduced ability to promote root system development [37].

Dobbelaere et al. obtained evidence of bacterial IAA's role in the wheat root system [38]. They performed experiments with wild and mutant *Azospirillum brasilense* strains. The mutant was deficient in IAA production due to the mutation of the indole pyruvate decarboxylase gene (*ipdC*). Wild strain and IAA addition stimulated the root development in wheat seedling. However, the mutant strain inoculation did not induce any response to the treatment.

On the other hand, Patten and Glick evaluated the response of canola seedlings to the inoculation with wild and mutant *Pseudomonas putida* (i*pdC*-lower IAA producer) [39]. The wild strain increased in root length by 35–50% compared to the mutant strain. Idris et al. evaluated IAA production by a wild strain of *Bacillus amyloliquefaciens* and five mutant strains with reduced IAA production [40]. Afterward, *Lemna minor* seedlings were treated with extracellular filtrates to determine the PGP effect of bacterial auxins; the mutated variants showed a significant decrease in IAA production, and only the wild strain and two mutant variants induced changes in plant growth.

Spaepen et al. described the effect of the *A. brasilense* Sp245 IAA producer strain on the root architecture of *A. thaliana* [9]. The inoculation of the wild strain induced the growth reduction in the seedling's main root and allowed for the proliferation of lateral roots. Increases in the root and shoot fresh weight were observed; such changes did not occur when the seedling was treated with a mutant variant of the strain (FAJ0009—knockout of the *ipdC* gene). The transcriptomic analysis of the root tissues of the plant showed that the wild strain induced increases in the expression of genes involved with the cell wall organization, tissue differentiation, organogenesis, induced systemic resistance, and the response to ethylene. These physiological responses have been associated with the action of auxins in plants.

Recently Duca et al. induced IAA overproduction in *Pseudomonas* sp. UW4 (WT-UW4), through its transformation with pRK415 plasmid, recombined with nit gene, which codifies nitrilase, an enzyme that converts indole-3-acetonitrile in IAA [41]. Canola seedling inoculation with mutant strain stimulated the lateral root production and increased roots length significantly regarding wild strain, this indicates the importance of the biosynthetic pathway indole–acetonitrile in IAA synthesis, and it revealed the IAA role in canola growth promotion by UW4 strain.

Although there is solid empirical evidence on the influence of auxin-producing bacteria on plant development, its role as a PGPR mechanism still raises some questions; for example, IAA production in the soil does not act independently; it is synergistic with other PGPR mechanisms [6]. Moreover, IAA production has been described as a virulence factor from plant pathology bacteria [15, 42]. IAA bacterial production is also a mechanism implicated in plant growth inhibition by deleterious rhizobacteria (DRB) [43].

Importance of IAA production for PGPR screening programs

Many PGPR prospecting programs are based on the isolation and selection of IAA-producing strains. However, some evidence provided in this review has shown that this bias does not guarantee successful or reproducible results with their inoculation under field or greenhouse conditions. Here, we present some examples of PGPR bioprospecting strategies based on IAA-producing strains selection. Etesami et al. proposed the choice of bacterial IAA production as the best trait for PGPR strains selection for biotechnological purposes; they concluded that a simple screening method to detect endophytic and rhizosphere IAA-producing bacteria is more efficient, economic, and reduces the probability of leaving out candidates with biotechnological potential [14].

Seven bacterial strains of the genus *Acinetobacter* spp., previously isolated from banana crops in Indonesia, were selected for their ability to produce IAA. The increase in IAA production was optimized by replacing tryptophan with mung bean, sprout extract, and fish meal

[44]. Pereira et al. screened cultivable bacteria with plant growth activity from the sugarcane rhizosphere under various levels of drought stress, proposing that the IAA bacterial production is an essential criterion for PGPR strains selection and concluded that IAA producer bacteria in soil could stimulate plant growth, even under stress conditions such as drought [45]. Genera *Acidovorax* and *Pseudomonas* were the main IAA producers.

Variability of IAA production across bacterial isolates

One of the factors that can influence the variability of the results with the inoculation of PGPRs under field or greenhouse conditions is the significant variation in the IAA bacterial production, even between isolates of the same species. Sridevi and Mallaiah evaluated the IAA production by 26 strains of *Rhizobium* sp., isolated from nodules of *Sesbania sesban* (L.) Merr. All the strains were positive for the qualitative detection of IAA, but the quantification allowed to establish that the amount of IAA was different between strains despite coming from the same nodules type and geographic zone. IAA production among the 26 strains was also differed depending on the type of carbon and nitrogen source type [46].

Gilbert et al. isolated 42 endophytic bacterial strains from duckweed tissues. Based on colorimetric and chromatographic evidence, they reported the production of different types of indole compounds by the strains and found a significant correlation between the indole type and the duckweed genus from which the bacterial strains were isolated. IAA bacterial production correlated only with isolates of some duckweed genera [47]. Therefore, this trait does not necessarily define the effectiveness of a strain as PGPR. This trait is neither an adequate filter to select candidate strains to develop biofertilizers.

Wagi and Ahmed selected 2 strains of *Bacillus* spp. with PGPR activity, and IAA production was one of the traits. However, its production was different between both strains, even under the same culture and incubation conditions [48]. This variability makes it difficult to choose IAA production as a selection trait for promising PGPRs.

Environmental modulation of IAA production in bacteria

The dependence of bacterial IAA synthesis on environmental conditions could also partially explain the variability of the results with the PGPR inoculation in planta. Sarwar et al. evaluated IAA production in 19 different soil samples. They determined in controlled experiments that IAA production by soil microbiota depends not only on tryptophan concentration, but also on environmental factors such as glucose and nitrogen concentration, pH, temperature, aeration, and incubation time. The effect of these variables is greater than the soil's physical and chemical properties on the IAA production by the microbial population [107].

Ona et al. evaluated the IAA production in vitro by *A. brasilense* Sp245 in a batch reactor (5 L), variating some environmental conditions. The experiments showed a maximum production of IAA and the overexpression of the IPDC gene (indole-pyruvate decarboxylase) with the addition of 50 $\mu g.mL^{-1}$ of tryptophan, under microaerophilic conditions (approximately 5% dissolved oxygen) for 18 h, other culture conditions (oxygen and tryptophan concentration) were less efficient for IAA production [49]. Sridevi and Mallaiah showed differences in IAA and biomass production of 26 strains of *Rhizobium* sp. strains under different tryptophan concentration conditions, carbon source, and nitrogen source conditions. Some strains increased their IAA production with mannitol as a carbon source, and others reacted similarly with sucrose, galactose, or glucose. Regarding the nitrogen source, some strains increased their IAA production with KNO_3, $NaNO_3$, $NaNO_3$ or, $(NH_4)_2SO_4$, while other strains showed this result with organic nitrogen sources such as L-glutamic acid [46].

Hoffman et al. reported the isolation of endophytic bacterial strains of the *Luteibacter* sp. genus from the leaf tissue of tomato plants. The strains were higher producers when the tomato plants they were isolated from were previously exposed to phytopathogenic fungus *Pestalotiopsis* aff. *neglecta*, the bacterial IAA production could also be modulated by exposure to phytopathogens, and it may be a phytoprotection response [50].

Chandra et al. optimized the IAA production in vitro by three bacterial isolates with PGPR activity. The isolates CA1001 and CA2004 showed better IAA production at pH 9 (91.7 $mg.mL^{-1}$) and at temperature 37 °C (81.7 $mg.mL^{-1}$). Dextrose (1%) was the best carbon source for isolate CA1001, with an IAA production of 104 $mg.mL^{-1}$. Isolate CA2004 showed a better production of IAA at 1.5% and 1% beef extract as nitrogen sources, respectively. Isolate CA1001 showed 32 $mg.mL^{-1}$ IAA production at 0.5% nicotinic acid concentration. The CA1001 and CA2004 isolates were evaluated in three different plant models and showed variable results according to the plant model [51].

Through a trial with corn seedlings under hydroponic conditions, Karnwal evaluated the effect of root exudates on IAA production by the bacterial strains *Kocuria rosea* VB1 and *Arthrobacter luteolus* VB2. The exudation patterns (type of compound and concentration) significantly modulated the IAA production of the strains, which has interesting practical implications for its possible inoculation in soil [52].

Auxin response in plants: determinants of variation

Auxins lead to the formation of new tissues and plant organogenesis; when exogenous factors such as light patterns, temperature changes and irrigation regime synchronize with endogenous factors such as gravitropic responses or changes in plant phenology, they induce the formation of new tissues that facilitate the adaptation of plants to such changes.

Auxins are accumulated in individual cells or small meristematic cell groups out of emergent plant tissue as a response to these stimuli; simultaneously, the rest of the auxin content migrates in the opposite direction to the tissue growth (Fig. 2); in this way, the response of auxins is triggered in the tissue in formation [53–55]. PIN (PIN-FORMED proteins) transport proteins regulate the concentrations of auxins inside the cell and facilitate their entry into and exit from the interior of endosomes, as well as their exchange within the extracellular medium.

When the required amount of IAA accumulates, it reaches the nucleus of the plant cell and induces the repressor peptide degradation (AUX/IAA), which regulates the transcription of the genes in charge of coding the auxin response factors (ARF) (Fig. 2) [55, 56]. Specific amounts of auxins in growing tissues or during organogenesis act as "adhesion" factors between the AUX/IAA repressor proteins and one ubiquitin, the latter transporting the repressor complex to the 26S proteasome, which degrades the repression and releases the expression of ARF genes.

The ARF expression regulates various processes of plant growth and development. The NPH4/ARF7 genes are expressed, which lead to the expression of a reporter gene in aerial and vascular tissues of primary roots; ARF19 is an active promoter in aerial vascular roots and tissues in seedlings, NPH4/ARF7 and MP/ARF5 are expressed during embryogenesis, HSS/ARF2 is related to seedling growth, while ARF6 and FWF/ARF8 aid in the formation of flowers [57]. ARF1 and ARF2 control leaf senescence and floral organ abscission. ARF3 and ARF4 participate in organ polarity specification. ARF3 also integrates the functions of AGAMOUS (AG) and APETALA2 (AP2) during floral meristem determinacy [58, 59].

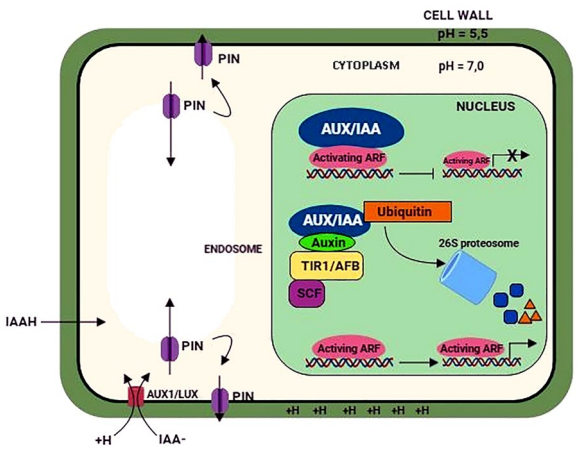


Fig. 2 IAA mechanism in the plant cell

In tomato plants, SlARF3 forms epidermal cells and trichomes; SlARF9 regulates cell division during early fruit development; SlARF7 is associated with crosstalk between auxin and gibberellin signaling; SlARF4 regulates the sugar metabolism during fruit development [58]. In rice, OsARF16 and OsARF12 regulate the iron deficiency response [58]. In addition, 50 ARF genes (OfARFs) were detected in *Osmanthus fragrans*, and many OfARF genes are associated with regulating flower developmental stages [60].

On the other hand, IAA sticks to the ABP1 membrane receptor, which activates a cascade reaction through a second messenger that enters the cell nucleus and induces the overexpression of the H+ATPase enzyme. Evidence shows the increase in activity of the preexisting H+ATPases in the cell due to the bond between IAA and ABP1. Both phenomena increase the pumping of H+into the apoplastic space allowing its acidification [61]. The apoplast acidification generates a loss in the rigidity of the cell wall, which facilitates its flexibility during root elongation and induces the active mitosis points emergence that becomes new secondary roots [26, 62]. The accumulation of H+also changes the polarity of IAA through protonation, facilitating auxin transport between plant tissues [57].

Auxins act through a system of signal transduction. Such mechanisms are dependent on the concentration of the signaling molecule, which indicates that, during their formation, each tissue requires a specific concentration of auxins to trigger the response of elongation and cell division. So, increments in the optimal concentration of auxins induce plant growth inhibition, and changes in its development patterns can be observed [63].

Endogenous factors of modulation

The metabolism of auxins is not entirely clear. The complexity of homeostatic processes generates a lot of difficulties with deterministic approximations of changes in the IAA concentrations and their derivatives at each stage of plant growth. However, some progress has been made, as explained below.

Variability in endogenous auxin activity by plant species

Some publications have described the differential effect with the application of exogenous auxins in mono and

eudicot plants. Masuda made an interesting experimental approach to the effect of IAA on tissue models of monocot (oat coleoptiles) and dicot (azuki epicotyls) plants. The author proposed that if the effects of auxin activity are observed in cell elongation and division, it is necessary to know the cell wall composition of both models before and during IAA-induced elongation. In this way, it would explain the differential response of both models to auxins. The uronic acid found in hemicellulose is considered a labile compound. Chromatographic patterns and methylation analysis results show that the oat coleoptile cell wall contains a small amount of xyloglucan in hemicellulose, auxins decrease the glucans content, and it causes an increase in arabinose and xylose. The rapid cell extension induced by auxins in monocots could be due to the breakdown of interconnections between cellulose microfibrils. The data obtained from epicotyls of azuki showed that approximately 80% of the xyloglucan chains linked by hydrogen with cellulose microfibrils are linked to rhamnogalacturonan, in dicots, the main constituents of hemicellulose are xyloglucans and galactans. At the same time, rhamnose is found only in pectin compounds [64].

Usually, eudicot plants are more sensitive to exogenous auxins and respond to lower hormone concentrations than monocot plants; this is the operating principle of auxinic herbicides. The explanation for this phenomenon has been hypothesized in three points: 1) the transport of auxins during embryogenesis is essential for plant development that defines the differentiation of the tissues. In this way, two cotyledons facilitate the auxins transport, which increases their sensitivity; 2) the reticulated vascularization of the leaves of dicot plants facilitates the distribution of the auxins and allows the plant to respond to small hormone doses, in comparison with the parallel vascularization of the monocot plants; 3) the root architecture in dicot plants consists of a primary root from which lateral roots emerge, while in monocot shoot-borne, adventitious roots are the predominant phenotype; this implies a fibrous constitution, which is less sensitive to exogenous auxins and leads to the degradation of auxins before its activity starts [65, 66].

Due to the above, monocot and dicot plants probably respond differently to inoculation with auxin-producing bacteria since some evidence indicates that the inoculum concentration emulates the "dose effect" of the exogenous application of auxins in plants [67]. Some exploratory tests developed in our laboratory show that maize seedlings (monocot) respond phenotypically to doses from 1–10 ppm of IAA (Fig. 3), while in eudicot models as cucumbers, they respond to doses from 0–1 ppm (Fig. 4).

In this way, exploratory assays with these plant models, but applying several inoculum concentrations of auxin producer PGPR strain *Lysinibacillus* sp. PB211, has allowed detecting differential effects in plant biomass production in both plant types according to inoculum concentration. These experiments were done in greenhouse conditions using sand as plant growth substrate, and plant mineral nutrition was supplied with Hoagland solution during irrigation.

Maize plants consistently presented (after three repetitions of the test) significant increases in biomass production with the inoculation of 1E8 spores per mL of PB211 (Fig. 5), while in cucumber this result was obtained with the inoculation of a concentration of 1E4 spores per mL (Fig. 6). Suarez et al. described a directly proportional relationship between the inoculum concentration of a bacterial strain producing auxins and the expected effect of auxin excess on plants [67]. On the other hand, these results agree with the previously described regarding the sensitivity of mono and dicot plants to exogenous auxins.

Variability by developmental stage

In vitro studies with corn grains allowed to know the endogenous mobility rate of auxins (homeostasis) from the seed towards the emerging tissues during germination in corn through the radioactive isotope dilution technique. During germination, conjugation, hydrolysis, and the mobility from the endosperm towards the roots and leaves regulate changes in the concentration of available IAA. In this way, the concentration of endogenous IAA varies according to the growth stage of the plants. This document also provides evidence of the effect of the exogenous addition of auxins and light stimuli on endogenous IAA homeostasis [68].

Dann et al. found short-time variations in the endogenous IAA concentration in branches of *Prunus persica* (L.) Batsch, which was consistent with changes in the diameter of branches [69]. These results indicate the relationship of plant growth with changes in the basipetal transport of endogenous auxins. Kobayashi et al. measured the IAA concentration in the leaves and ears of Oryza sativa L. cultivar Nihonbare (japonica variety) plants throughout their entire growth cycle. The IAA content in the ears was higher than in the leaves. However, the proportions of free and conjugated IAA changed according to the growth stage. At the beginning of the tillering stage, there was a significant decrease in the conjugated IAA in leaves, and the free IAA remained stable and superior until the initiation of the panicle. There was a significant increase in the conjugated IAA in the ears from the heading stage to the anthesis, while the free IAA decreased. After this stage,

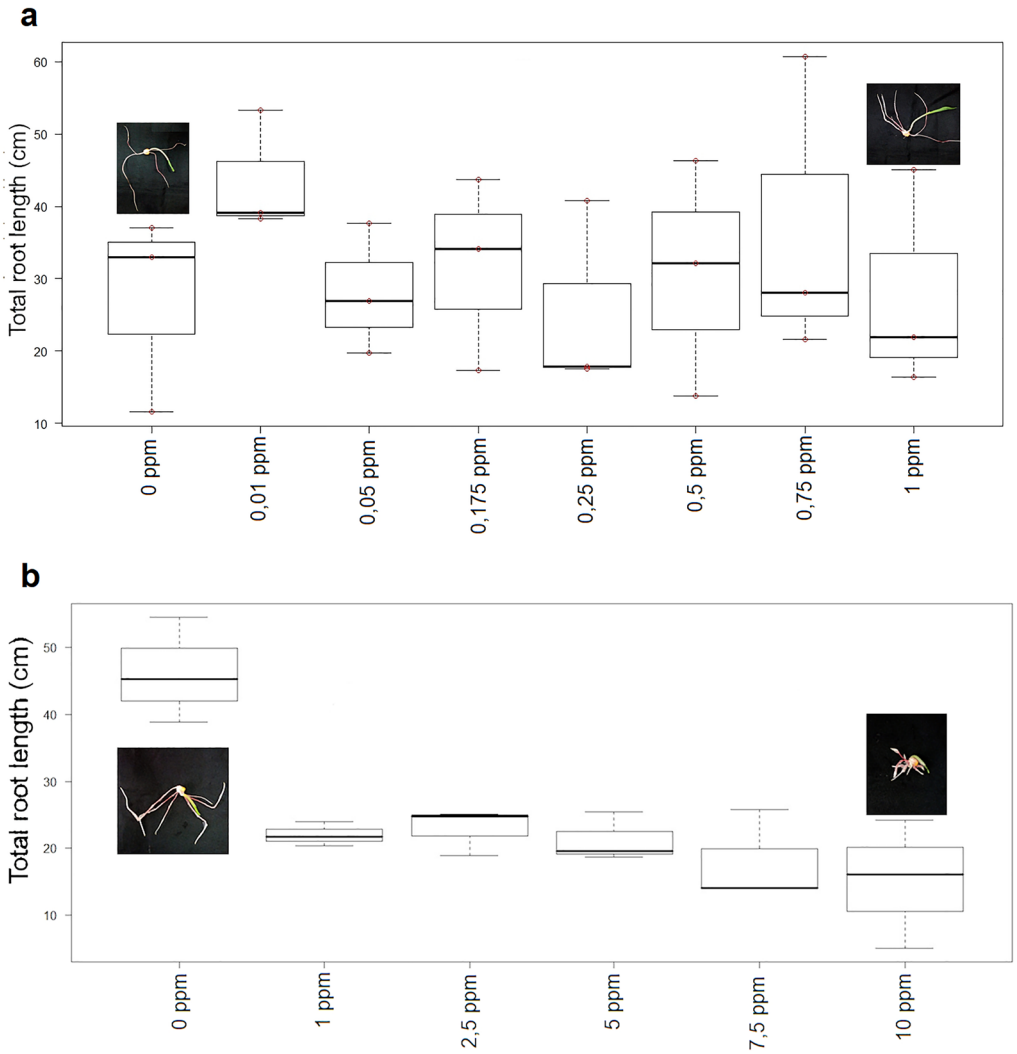


Fig. 3 Effects of IAA on the root of maize seedlings

both types of IAA decreased [70]. These results provide evidence of the fluctuation of IAA concentrations for each stage of plant growth. Nordstrom and Eliasson evaluated the content of IAA and IAAsp (IAA conjugated with the amino acid aspartic acid) in various parts of pea leaf cuttings (*Pisum sativum* L. cv. Marma) during the formation of adventitious roots. From day 0 to day 3, there was a significant increase in IAAsp levels in the intact plant and a reduction in IAA levels in the internodes 4 and 5 of the cuttings. A decrease in IAA level in the root regeneration zone was achieved by eliminating the shoot apex, resulting in almost complete inhibition of root formation. The conjugation controls the accumulation of IAA in the tissue with Asp, and basipetal transport mobility from the apex root to the root's tip occurs [71]. Gokani and Thaker provided experimental evidence for the role of endogenous auxin production on cotton fiber length in three different cultivars; the content of free and conjugated IAA varies over time and between them. They also described a positive correlation between auxin content and fiber length. This correlation also varies between cultivars. The type of auxin (synthetic or natural) in in vitro experiments also showed an effect on the length and weight of the fibers [72].

External factors of modulation

Effects of light on changes in endogenous auxin concentration

Light is an environmental factor regulating plants' IAA synthesis, transport, and homeostasis. The FIN219 gene encodes a protein similar to the GH3 family proteins, and auxins induce its expression. In addition, this gene interacts with another signaling component of phytochrome

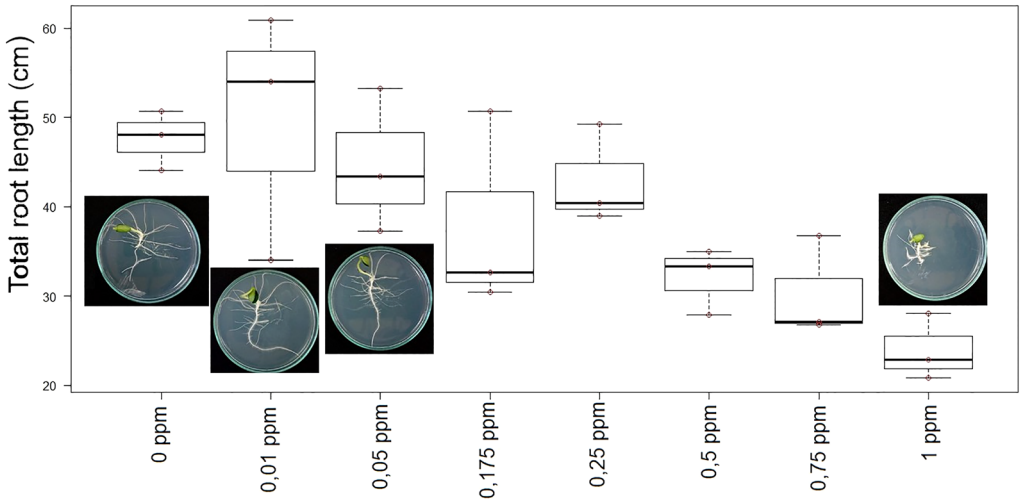


Fig. 4 Effects of IAA on the root of cucumber seedlings

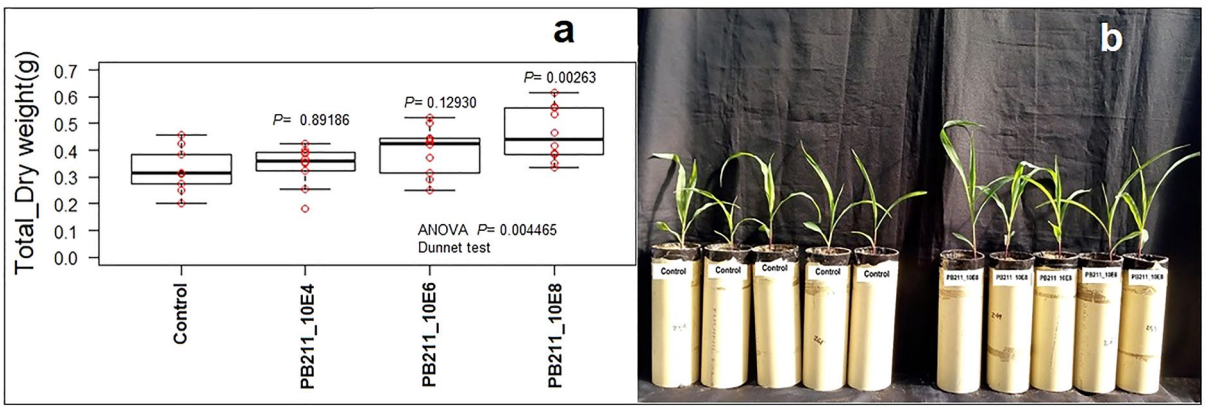


Fig. 5 Effect of Lysinibacillus sp. PB211 on maize plants

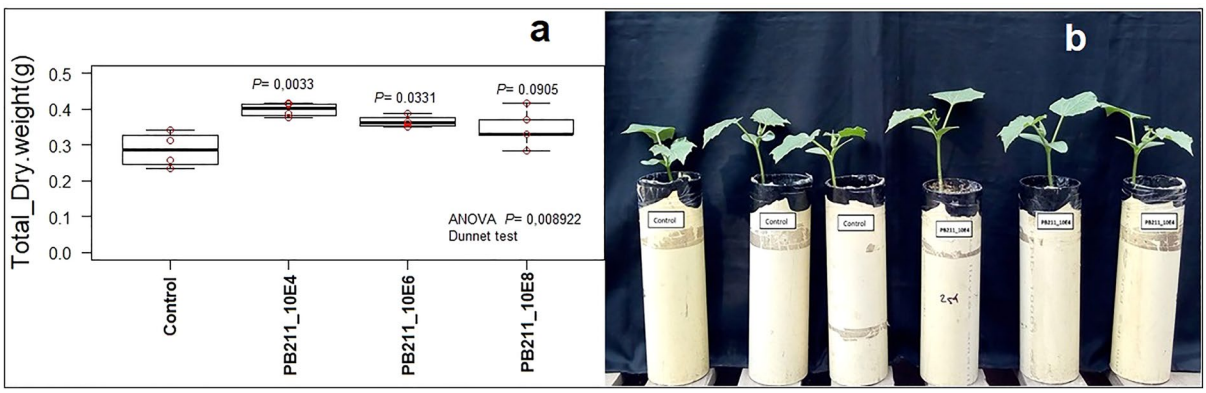


Fig. 6 Effect of Lysinibacillus sp. PB211 on cucumber plants

A; this gene regulates plants' response to the far-red spectrum of light. The FIN219 mutant exhibited an elongated hypocotyl phenotype with the far-red spectrum [73].

Hoecker et al. described the red-light spectrum effect on IAA homeostasis in *Arabidopsis thaliana* (L.) Heynh plants. The mutation in the red1 receptor-induced defects in cytochrome P450 CYP83B1. Therefore, the production of indole glucosinolates from the indole-3-acetaldoxime (IAOx) pathway decreases and consequently increases IAA synthesis. Plants with overproduction of IAA acquire an ethyloated phenotype [74]. Sorin et al. used Arabidopsis thaliana mutants better to understand the physiological and molecular basis of adventitious rooting. The *argonaute1* (*ago1*) mutants show almost complete inhibition in adventitious root formation. The defect in adventitious rooting observed in ago1 was correlated with hypersensitivity to light (due to overexpression of phytochrome A- *phyA*), and an apparent decrease in endogenous levels of free and conjugated IAA was shown. The reaction of all *ago1-3* mutants was differential for each light spectrum evaluated, but the phenotype was permanently stunted. The ARF17 gene, auxin-inducible gene repressor, was overexpressed in hypocotyls *ago1-3*. The overexpression of ARF17 showed less production of adventitious roots than the wild type, and it conserved a lower expression of GH3 genes [75].

The COP1 protein (CONSTITUTIVE PHOTOMORPHOGENIC 1) is a type of ubiquitin ligase. It is a central integrator of photoreceptor responses through CSN recognition (the COP9 signalosome); subsequently, COP1 is degraded by the 26S proteasome. Light through COP1 modulates root and shoots growth in *Arabidopsis*. COP1 regulates auxin transport from shoot to root by controlling the transcription of the PIN-FORMED1 auxin output carrier gene (PIN1). In this way, the levels of auxins derived from the shoots to the roots are adjusted. In addition, the intracellular distribution of PIN1 and PIN2 in the root is dependent on COP1, which facilitates the adjustment of root growth [76].

The tip of the root plants shows positive gravitropism; this behavior is governed by the root cap. Suzuki et al. experimentally demonstrated the role of auxins and their light dependence on modulating root growth. Corn seedlings exposed to light showed the typical reaction of positive gravitropism ("U" curvature of the root tip). Decapitation (removal of cap root) of the root tip generated roots without curvature. The exposure of seedlings to light generated a higher content of endogenous root auxins than plants in darkness. The effect of light on root endogenous auxins content was reverted by decapitation of the root tip. The treatment of light-exposed seedlings with chemical auxin blockers inhibited the curved phenotype. The overexpression of the Zmvt2 and Zmyuc genes suggests that IAA accumulation in the transition zone is due to light-induced activation [77].

Effects of water availability on endogenous auxin activity

Pustovoitova et al. evaluated the effect of water stress on IAA content in *Cucumis sativus* L. leaves. IAA concentration was higher in irrigated plants from the first days of the experiment. After day 4, both treatments (with and without irrigation) showed a decrease in the levels of IAA in the leaves. However, on day 15, the IAA levels in the soil without irrigation were significantly lower. Abscisic acid concentrations also modulate the homeostasis of IAA under dryness conditions [78].

In experiments performed with rice (japonica rice Zhonghua 11—*Oryza sativa* subsp. *Japonica*), Xiushui 11 (XS11), indica rice Zhenshan 97 (ZS97), Minghui 63 (MH63), 9311, IR64, and ZH11 some observations were made related to various abiotic stress stimuli. The endogenous content of IAA decreased under drought stress conditions but increased gradually under heat and cold stress. Several IAA biosynthetic and pathway-signaling genes showed changes in their transcription under these conditions, and these transcription changes were correlated with endogenous IAA concentrations [79].

In an experimental system with mutant plants (DR5:GUS), it was observed that drought stress significantly decreased auxin activity in Arabidopsis plants. Mutants yuc1, yuc2, and yuc6 with lower endogenous IAA levels showed decreased stress resistance compared to wild-type plants. The viability of the plants was restored with the exogenous addition of IAA. The resistance of plants to drought stress is significantly modulated by auxin activity. Additionally, the endogenous hormone content is regulated by water availability in the soil [80].

Auxins have a direct effect on the resistance of plants to drought stress. The addition of IAA significantly increased the dry weight and relative water content of clover plants under drought stress. This effect was reversed by adding an IAA inhibitor (L-AOPP). In addition, treating IAA in plants under drought stress increased the endogenous content of abscisic and jasmonic acid. Exogenous IAA treatment also up-regulated the auxin response genes (GH3.1, GH3.9, IAA8) and stress response genes (bZIP11, DREB2, MYB14, MYB48, WRKY2, WRKY56, WRKY108715, and RD22) [81].

Changes in nutrient availability modulate endogenous IAA in plants

Bates and Lynch evaluated the effect of phosphorus (P) variation on root hair elongation in *A. thaliana*. Plants in P starvation (only supplemented with 1 mmol of P) presented a phenotype with significantly elongated root hairs compared to plants supplemented with sufficient

phosphorus (1 mol). These results were attributed to an auxin signaling response, which induces the emergence of root hairs and stimulates their elongation. Hence, the plants compensate for the lack of phosphorus by seeking new sources. The mutant *A. thaliana*—axr2 (low concentration of auxins in the roots) did not present the elongated phenotype under P starvation conditions, but this was restored by adding exogenous auxins [82]. The mutant *A. thaliana—axr2* (low concentration of auxins in the roots) did not present the elongated phenotype under P starvation conditions, but this was restored with the addition of exogenous auxins. The role of auxin on root architecture modulation under P starvation was also proven through tests with mutant *Arabidopsis thaliana DR5:uidA* plants [83].

Pérez-Torres et al. developed a theoretical model based on a transcriptomic experimental approach of A. thaliana plants growing under conditions of phosphorus deficiency. The authors observed that under conditions of inorganic phosphorus (Pi) deficiency, there is an increase in the expression of TIR1 (Transport Inhibitor Response1), which induces the degradation of the repressor AUX/IAA and triggers the expression of the auxin response factor ARF19, which is responsible for the modulation of the root architecture. In this way, they concluded that phosphorus deficiency increases the sensitivity of plants to auxins [84]. Miura et al. contributed to the refinement of the theoretical model of auxin regulation in A. thaliana under conditions of phosphorus deficiency. They provide evidence that SIZ1 (E3 SUMO-protein ligase SIZ1-transcriptional activator controlling the phosphate deficiency responses) participates in the regulation of auxins activity to modulate root system architecture in response to Pi starvation. siz1 causes the typical phenotype in Pi starvation: the inhibition of primary root (PR) elongation and the promotion of lateral root (LR) formation. Although mutations in siz1 caused the opposite phenotype (more significant PR growth inhibition and LR development), this response was also obtained in wild plants supplemented with IAA under Pi starvation [85].

The APSR1 gene (Altered Phosphate Starvation Response1) modulates the expression of some changes in root architecture under Pi starvation conditions. The mutation of this gene in *A. thaliana* results in an indifferent response to the Pi concentration in root architecture variables. There is an indirect relationship between the expression of APSR1 and PIN7 auxin transporters functioning, which are less expressed in APSR1 mutant plants. Because of its structure and subcellular localization, APSR1 probably acts as a transcription factor for PIN7 accumulation at the root tip [86].

Walch-Liu et al. summarized the mechanisms of changes in root architecture in nitrogen-deficient media. In addition, they explain the role of the ANR1 gene and its effect on the regulation of auxin transport from leaves to roots. Thus, soil nitrogen deficiency also affects plants' endogenous auxin homeostasis [87].

Tian et al. evaluated the effect of fertilization with increasing nitrate concentrations on root architecture, endogenous nitrogen content, and endogenous auxin content in maize plants. Nitrates inhibited root growth, and this was due to a decrease in cell elongation and not due to changes in the length of the meristems. The IAA concentration in the phloem exudates was decreased with high concentrations of nitrates. Exogenous naphthalene acetic acid (NAA) and IAA restored the elongated phenotype of the primary root at high nitrate concentrations [88]. The auxin response of plants to nitrates availability is regulated by the presence of glutamine/glutamate or their ability to synthesize them. In an experiment, Gifford et al. chemically blocked glutamine/glutamate synthesis in *A. thaliana*; consequently, the activity of the auxin response factor ARF8 and its 126 possible targets were repressed. ARF8 expression was subsequently restored with the addition of glutamine [89].

Effects of exogenous auxin activity on endogenous IAA homeostasis in plants

Some evidence indicates that the exogenous IAA activity in roots changes the endogenous homeostasis of IAA in plants. Inoculation of wheat plants with auxin-producing PGPR strains induced significant increases in endogenous IAA synthesis. In addition, there was a positive correlation between the bacterial production of auxins and endogenous synthesis in plants of *Vigna radiata* [90]. Applying 100 $mg.L^{-1}$ C of humic acids extracted from leonardite under hydroponic conditions in a growth chamber induced significant increases in IAA synthesis in leaves and roots of *Cucumis sativus* L. cv Ashley [91].

Cai et al. studied the variations in endogenous hormonal content in two wheat varieties by adding exogenous hormones under field conditions. The addition of exogenous IAA induced a significant increase in the concentration of endogenous IAA in shoots from the second day after the treatment. The addition of exogenous IAA negatively correlated with biomass production and tiller length [92].

Auxin-like activity of soil

The soils able to harbor vegetation naturally contain specific concentrations of auxins, which induce the hormonal activity of soil on the development of plants; these auxins come from the microbial activity of soil as well

from root exudation [22, 63, 107]. Some early reports since 1942 described the detection and quantification of bioavailable IAA in forest pristine soil samples and degraded soils from North America, a direct relationship between soil fertility and IAA content was observed [20]. Afterward, Frankenberger and Brunner described a methodology for analytical quantification of IAA in soil samples by HPLC (high-performance liquid chromatography); the detection of some intermediate compounds, such as indole acetamide acid and indole pyruvic acid, led to the deduction of the metabolic pathways used by soil microbiota for IAA synthesis [21]. More recently Szajdak and Maryganova quantified IAA contents on soil samples from Poland, and they found a direct relationship between the IAA concentration and humic substances content in the soil. Additionally, soils previously treated with peat had a higher content of IAA and humic substances [22].

On the other hand, humic substances have a biological reactivity type, which exerts a hormonal effect of the auxin type called the "auxin-like effect"; such phenomenon is explained due to their chemical heterogeneity and the distribution of the hydrophobic and hydrophilic domains [24, 24–26, 62, 93, 94]. This has been described by (de Sanfilippo et al., 1990), who suggest that, due to this effect, the application of high amounts of humic acids in plants is restrictive for their development [95].

Canellas et al. described the bioactivity of humic acids obtained from bovine manure vermicomposting; the addition of some concentrations of humic acids induced increases in length, surface area, and the number of lateral roots of maize seedlings in a soil-free experimental system; an increase of the H + ATPase enzyme activity was also observed, such enzyme activity enhances the flux and gradient of hydrogen ions in the apoplastic space, which is associated with rooting elongation, this effect is similar to the one obtained by adding IAA in plant roots [23]. Furthermore, interchangeable auxin groups (as IAA) were also detected by structural chemical analysis of the humic acids from the compost. These results are solid evidence of the hormonal activity attributed to humic acids. This last finding agrees with recent reports indicating that some labile fractions can disintegrate from the humic suprastructure and interact with the root surface or enter the root tissues [24, 93, 96–98].

Canellas and Olivares [26] and Canellas et al. [25] displayed experimental evidence that correlates the hormonal activity of humic acids with changes in exudation root patterns, which implies changes in the rhizosphere microbial communities selection; such results suggest that the PGP effect of some rhizobacteria might depend on the soil humic content. It implies the need to explore this topic in the design of new biofertilizers.

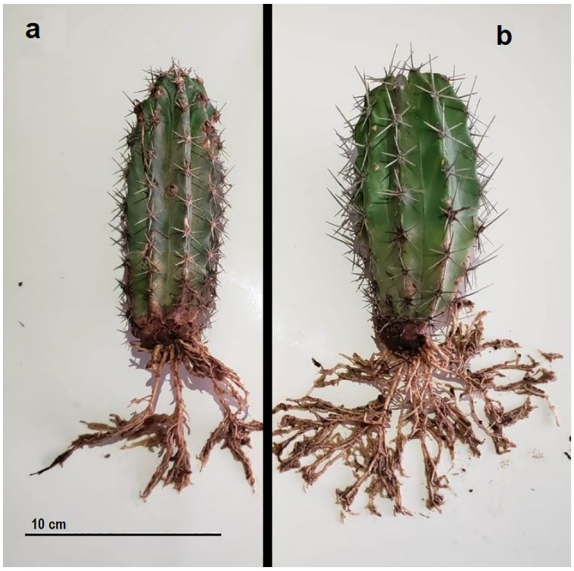


Fig. 7 Effect of humic acids on cactus plants root system

These results agree with Valero et al. who detected chemical groups similar to IAA in humic acids coming from bacterial solubilization lignite [99]. Then, Valero et al. provide evidence of auxin-like effect of these humic acids [100]. Taborda and Valero (2019-unpublished) found the effect of humic acids on the rooting of cactus plants in desert areas of Colombian Guajira (Fig. 7), this effect is attributed to the auxin-like activity of humic acids.

Although the bioactivity of humic substances has also been associated with effects type cytokinin [108, 109, 113], abscisic acid—ABA [110, 110], alkamides [111], NO [112], gibberellic acid [25, 113], and the enzymatic homeostasis of reactive oxygen species [93], the hormonal mechanism of humified organic matter with more available evidence is the so-called auxin-like effect [93, 113].

Sum of effects: the soil–plant–bacteria system and the consistency of IAA-producing PGPR.

Sum of "auxin-like" effects

Auxins trigger a series of reactions associated with plant growth, especially during organogenesis and the emergence of new tissues; for example, during the proliferation of secondary roots, which increases the absorption of nutrients by the plants and facilitates their adaptation to soil conditions; however, this effect is mediated by specific concentrations of auxins, and the excess of such molecules has a deleterious effect on plant development.

Although IAA production is a PGPR mechanism, their inoculation at inadequate concentrations during periods of high auxin production by plants could be

deleterious for plant growth. Puga-Freitas et al. evaluated the growth and transcriptomic of *A. thaliana* (WT col-0) and a mutant version of it (aux1-7/axr2-4), deficient in auxins production, with less IAA content in the root in soils with 2 levels of auxin-like activity by addition of earthworms. Mutant plants improved their growth and increased gene expression related to biotic and hormonal induction, cell structure, and differentiation; on the other hand, wild plants showed attenuated growth; this result was attributed to excess auxin content in soil–root interaction [101].

Suarez et al. evaluated the effect of the inoculation of *Micrococcus luteus* (high auxin producer) on *A. thaliana* at three concentrations (10^5, 10^6, and 10^7 CFU mL^{-1}), to classify it either as a deleterious rhizobacterium (DRB) or as PGPR. The combined application of the highest dose of the bacterial strain and earthworms (*Aporrectodea caliginosa*) had a deleterious effect on the growth of *A. thaliana* [67]. These results agree with an increase in the concentration of IAA in the soil of the experimental system with the application of these treatments. Therefore, it is possible that the interaction of PGPR strains with the high production of IAA under high auxin activity conditions in the soil can have a negative effect on plant growth. Although, in this case, the authors did not discuss the effect that earthworms have on auxin activity in the soil, the processing of soil organic matter by these organisms generates compounds with auxin activity, as shown by Canellas et al. [23], and Puga-Freitas et al. [101].

Recently, Blouin suggested that auxin content acts in signaling within the chemical communication process between soil and plants. It is evidence of the coevolution between plants and other soil organisms [102]. In this way, some bacteria could hurt plant development if they are inoculated in soils with high auxin activity and plants with high levels of auxins in root tissues.

Considering that most bacteria isolated from the rhizosphere can produce auxins, that plant auxin endogenous content varies according to the species and the phenological stage of development, and the soil has auxin activity, it is necessary to explore the effect of this auxin context on PGPR activity to enhance the production of biofertilizers. These topics show the need to consider the characteristics of the soil and its humic content before applying PGPR inoculants. For example, in some soil types, it might be convenient to inoculate selected strains by expressing other mechanisms different from the production of IAA or low production. This consideration is important because PGPR strains with high production of IAA are associated with positive effects in promoting plant growth. However, this may not be true in all cases.

One of the biggest challenges in producing biofertilizers is achieving the reproducibility of field results; the literature review allows for the detection of inconsistencies based on the knowledge gaps of PGPR mechanisms and the conditions in which they are evaluated in the field. In this way, the total auxin level in the PGPR–plant–soil relationship is a topic thus far unexplored in the design and application of biotechnological products of agricultural interest.

Effect of IAA sum on other PGPR mechanisms

Many PGPR strain screening programs for biofertilizer development are based on selecting a particular trait. However, the bacterial production of auxins is usually linked to the expression of other mechanisms. In this way, due to the dramatic effects of the bacterial production of IAA on plant growth, it is reasonable to propose screening programs that always consider the bacterial production of IAA regardless of the trait pursued. Here are some examples.

Glick et al. evaluated the effect of the strain Pseudomonas putida GR12-2 and its mutant version GR12-2/acd68, with deficiencies in ACC deaminase activity. The wild strain presented a marked PGPR effect on variables such as length, dry weight, and dry weight of the roots in canola seedlings. However, the mutant version of the strain presented harmful effects. In some cases, it presented lower growth than the control. The authors speculatively explained this result as follows: a reduction in ACC deaminase activity induces an increase in the concentration of ACC (1-aminocyclopropane-1-carboxylic acid), a precursor of ethylene (plant growth inhibitor), and it is synthesized from IAA [103]. Therefore, an imbalance in the bacterial production of IAA and ACC deaminase activity could harm plants.

Duca et al. [41] provided evidence of the approaches of Glick et al. [103]. They evaluated the effect of the overexpression of IAA synthesis of the strain Pseudomonas sp. UW4 on its ACC deaminase activity. They used mutants that overexpress the activity of genes of the IAA biosynthetic pathway from UW4 (IAM pathway). All transformed strains increase the bacterial production of IAA and significantly reduce ACC deaminase activity. IAA is a precursor to the synthesis of ACC; this, in turn, is a precursor to ethylene synthesis. In this way, the overproduction of IAA alters the IAA–ethylene feedback regulation, which could affect ACC deaminase activity.

B. subtilis LK14, isolated from Moringa peregrina bark, was selected among several endophytic strains for its phenotypic traits associated with PGPR activity (high

ACC deaminase activity and high IAA production). Although this strain presented an apparent PGPR effect in tomato plants, the authors suggested a positive and complementary interaction between both traits [104].

Bacillus amyloliquefaciens FZB45 showed the ability to promote Chinese cabbage growth due to its phytase activity in high concentrations of phytates. Additionally, FZB45 directly affected plant growth, probably due to IAA production. The bacterial production of auxins creates a response dependent on the inoculum concentration; therefore, the authors suggest an interaction with the effects mediated by phytase [105].

Chaiharn and Lumyong screened bacterial strains with PGPR potential from rhizosphere agricultural soil. They selected the strains based on their ability to produce IAA and to solubilize inorganic phosphate in vitro. Based on these results, the authors suggest a combined effect of both mechanisms on the significant increase in the length of adventitious roots of bean plants [106]. The effect of exogenous auxins and variations in the concentration of inorganic phosphorus on the root architecture has been previously reported.

Conclusions

We propose reevaluating the production of IAA as a trait to define the effectiveness of a bacteria as PGPR. Although bacterial IAA production by PGPR causes positive effects on plant growth, it is difficult to reconcile this effect with the rest of the auxin-like factors, such as plant, soil, and microbial populations in the rhizosphere.

In the first stage, we mentioned some research works that reported the IAA production as a selection trait for the PGPR isolation; we observed some inconsistencies in the results obtained and displayed the inconvenience of this trait as a selection factor. Later, some environmental factors that modulate the IAA production by bacteria were explained. We highlighted that the variability in IAA bacterial production is a modulating factor of the results of the PGPR inoculation *in planta*.

On the other hand, some factors that determine the variation of the endogenous auxin response of plants were addressed. Factors such as plant species, growth stage, light type and intensity, water availability, nutrient availability, and exposure to exogenous auxin sources in the rhizosphere modulate endogenous auxin synthesis and signaling in plants. Therefore, these factors may also affect the effectiveness of IAA-producing bacteria inoculation in plants.

Finally, this work shows the role of soil auxin-like activity, especially from humified organic matter, on plant growth and suggests that this effect can also modulate the PGP activity of IAA-producing bacteria. Therefore, the sum of auxin-like effects: IAA bacterial production, endogenous auxin signaling in plants, and the soil auxin-like effect, as well as all the possible variation factors in each of them, can potentially affect the effectiveness of biofertilizers and biostimulants in agriculture.

In this way, this manuscript presented critical reasoning about the importance of auxin interaction in PGPR formulation and application. Moreover, this highlights the need to do trials to elucidate biochemical, ecological, and evolutionary topics in the soil–plant–bacteria relationship to obtain better practical biotechnological applications.

Acknowledgements
Not applicable.

Author contributions
The manuscript was prepared by MP-G. Figures 1 and 2 were designed by MP-G; Figs. 3, 4, 5, and 6 were generated from preliminary results by MP-G and CR; Fig. 7 was provided by NV-V. All authors read and approved the final manuscript.

Funding
This work was written with the support of the Ministry of Science and Technology of Colombia (Grant number 757 of 2016-Colciencias National Doctorates). Researcher granted: Manuel Pantoja-Guerra.

Availability of data and materials
The datasets used during the current manuscript are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 28 August 2022 Accepted: 5 December 2022
Published online: 27 January 2023

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