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Crescimento radicular em plântulas de tomate em resposta a inoculação da bactéria *Serratia* sp.

Root growth in tomato seedlings in response to bacterial inoculation *Serratia* sp.

Crecimiento radicular en plantas de tomate en respuesta a la inoculación de S*erratia* sp.

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Resumo

As rizobactérias promotoras de crescimento são bactérias do solo que habitam o entorno da raiz, e estão direta ou indiretamente envolvidas na promoção do crescimento e desenvolvimento das plantas. A eficiência da produtividade desses grupos de microrganismos pode ser aplicada ao plantio de culturas, constituindo uma alternativa interessante, para minimizar os efeitos negativos do déficit hídrico. O objetivo deste estudo foi verificar se o mecanismo de promoção do crescimento da bactéria é semelhante ao promovido pelo Polietileno glicol (PEG) e comparar os possíveis efeitos do estresse hídrico no tomate com os efeitos da inoculação da bactéria *Serratia* sp . A metodologia foi baseada em bioensaios *in vitro* utilizando plântulas de tomate (*Solanum lycopersicum* L.), mantidas em câmara de

crescimento com temperatura de 25 °C e fotoperíodo de 12 horas. Os resultados revelaram que a promoção do crescimento da raiz de tomate por *Serratia* sp. é semelhante ao promovido pelo PEG a 7%, diferindo significativamente dos resultados encontrados com diferentes doses de ácido indol-acético (AIA). A promoção do crescimento radicular em tomateiro por *Serratia* sp. e PEG 7% indicam parcialmente um efeito físico, uma vez que a restrição hídrica imposta pela molécula PEG diminui a capacidade de movimentação da água, também observada por bactérias, ao colonizar tecidos e células vegetais (biofilme), reduzindo a condutividade hidráulica da água através da raiz. O estímulo à promoção de crescimento radicular nos tomates não pode ser reproduzido pela auxina.

Palavras-chave: AIA; déficit hídrico; PEG; *Serratia* sp.; tomate.

Abstract

Growth-promoting rhizobacteria are soil bacteria that inhabit the surrounding root, and are directly or indirectly involved in promoting plant growth and development. The productivity efficiency of these groups of microorganisms can be applied to planting crops, providing an interesting alternative for minimize the negative effects of water deficit. The objective of this study was to verify if the mechanism of growth promotion of the bacterium is similar to that promoted by polyethylene glycol (PEG) and to compare the possible effects of water stress on the tomato against the effects of inoculation of the bacterium *Serratia* sp. The methodology was based on *in vitro* bioassays using tomato (*Solanum lycopersicum* L.) seedlings, kept in a growth chamber with temperature of 25 °C and photoperiod of 12 hours. The results revealed that the promotion of tomato root growth by *Serratia* sp. is similar to that promoted by PEG 7%, differing significantly from the results found with different doses of indoleacetic acid (IAA). The promotion of root growth in tomatoes by *Serratia* sp. and PEG 7% partly indicates a physical effect, since the water restriction imposed by the PEG molecule decreases the water movement capacity, also observed by bacteria, when colonizing plant tissues and cells (biofilm) reducing the hydraulic conductivity of water through the root. Stimulation to promote root growth in tomatoes cannot be reproduced by auxin.

Keywords: IAA; PEG; *Serratia* sp.; tomato; water deficit.

Resumen

Las rizobacterias que promueven el crecimiento son bacterias del suelo que habitan el ambiente de la raíz y están directa o indirectamente involucradas en la promoción del crecimiento y desarrollo de las plantas. La eficiencia de la productividad de estos grupos de

microorganismos se puede aplicar a la plantación de cultivos, lo que constituye una alternativa interesante para minimizar los efectos negativos del déficit hídrico. El objetivo de este estudio fue verificar si el mecanismo para promover el crecimiento de bacterias es similar al promovido por el polietileno glicol (PEG) y comparar los posibles efectos del estrés hídrico en los tomates con los efectos de la inoculación de la bacteria *Serratia* sp. La metodología se basó en bioensayos in vitro utilizando plántulas de tomate (*Solanum lycopersicum* L.), mantenidas en una cámara de crecimiento con una temperatura de 25 °C y un fotoperíodo de 12 horas. Los resultados revelaron que la promoción del crecimiento de la raíz de tomate por *Serratia* sp. es similar al promovido por PEG al 7%, que difiere significativamente de los resultados encontrados con diferentes dosis de ácido indol-acético (AIA). La promoción del crecimiento de las raíces en tomate por *Serratia* sp. y PEG 7% indican parcialmente un efecto físico, ya que la restricción de agua impuesta por la molécula de PEG disminuye la capacidad de movimiento del agua, también observada por las bacterias, al colonizar tejidos y células vegetales (biopelícula), reduciendo la conductividad hidráulica del agua a través de desde la raíz La auxina no puede reproducir el estímulo para promover el crecimiento de las raíces en los tomates.

Palabras clave: AIA; déficit hídrico; PEG; *Serratia* sp.; tomate.

1. Introduction

One of the alternatives showing beneficial effects in agriculture is the application of plant growth promoting bacteria (PGPB) or plant growth promoting rhizobacteria (PGPR). PGPRs are the soil bacteria that inhabit the root environment, and are directly or indirectly involved in promoting plant growth and development through the production and secretion of various regulatory chemicals. Generally, PGPBs directly facilitate plant growth by either assisting in the acquisition of essential mineral resources such as nitrogen and phosphorus or by modulating plant hormone levels. Indirectly, growth can be promoted by decreasing the inhibitory effects of various pathogens that act as biocontrol agents (Ahemad & Kibret, 2014).

Serratia bacteria are known for their ability to solubilize phosphate, siderophores and others. However, there are few studies that elucidate the production of phythormonium by these bacteria, such as the production of gibberilins by *Serratia nematodiphila* PEJ 1011. Kang et al. (2015) and indoleacetic acid (IAA) by *Serratia nematodiphila* NII-9228 described in studies by Dastager, Deepa & Pandey (2011).

According to Mohite (2013) the promotion of root growth is one of the main benefits attributed to bacteria that produce IAA. Rapid root establishment is very advantageous for young plants. The proliferation of root numbers enables a better anchorage of the plant to the soil, thus efficiently obtaining greater absorption of water and other mineral nutrients, enhancing their chances of survival.

The biosynthesis of IAA is closely related to the amino acid tryptophan and the tryptophan precursor indole-3-glycerol phosphate. Molecular genetics studies have been developed to elucidate the major tryptophan-dependent enzymes associated with IAA biosynthesis, as well as the order in which they act in this process. Biosynthetic routes using tryptophan as an IAA precursor in plants have also been identified in the bacterial pathway of IAA biosynthesis (Normaly, 2010, Taiz & Zeiger, 2013).

Understanding the different mechanisms that regulate plant growth promotion by PGPRs is the key to the complex plant-host interaction. The benefits promoted by morphological and physiological changes of the plant or even by competition, protecting the plant against pathogens, can be observed when colonization by these microorganisms occurs.

The promotion of bacterial-induced root growth through physical effect has not yet been mentioned in the literature, but the works by Asli & Neumann (2009, 2010a, 2010b) helps to explain the physical mechanism as a growth inducer in plants.

Asli & Neumann (2009) were the first to mention the physical effect as root growth promoter. For this, the aforementioned authors developed a laboratory test, aiming to evaluate the effect of bentonite clay-derived substances and TiO_2 nanoparticles, present in colloidal suspensions, on the root growth of corn (*Zea mays* L.) seedlings. The results found in this work revealed that these colloidal suspensions, once in contact with the roots, induced the reduction of water flow. The low water availability caused by these substances led to a rapid physical inhibition of water flow through the cell wall pores, which enhances root growth.

Similar results could be observed when Asli & Neumann (2010a) verified the mechanisms of growth promotion in maize when they were submitted to the presence of humic acids (HA). Humic substances (HS) are organic compounds derived from the oxidation and polymerization of organic matter. The presence of humified organic matter in soils may be associated with the transport of colloidal agglomerates or supramolecular solutions that directly influence the chemical, physical and biological properties of the soil.

Promotion of plant growth (bioactivity) induced by these organic substances in solution exerts a chemical stimulus on plant root architecture, such as increased availability of

mineral nutrients as well as the production of regulatory biomolecules (Braz, Canellas & Medici, 2010).

In addition to this chemical effect known in the literature, Asli & Neumann (2010a) were able to prove in their work with root growth of corn (*Zea mays* L.) seedlings that the increase in root development was also partly associated with a physical effect of humic acids.

The potentially stressful effects induced by colloidal solutions reveal that the clogged root pores caused by these polymers reflect the physical effect of growth promotion.

Therefore, this work aimed to test the hypothesis that PGPR could also promote plant growth by physical effect.

2. Material and methods

The plant material used in all experimental trials, for this laboratory research (Pereira, Shitsuka, Parreira & Shitsuka, 2018) , were tomato seeds variety Santa Clara Miss Brazil (*Solanum lycopersicum* L.).

Growth chamber adaptation

The experimental model developed *in vitro* in this work was based on previous laboratory tests with tomato seeds in 2011 and 2012. A BOD-like system was installed on the bench, consisting of 4 sets of fluorescent lamps with approximately 50 micromol m-2 s-1 photons, connected to a timer setting a 12-hour photoperiod, supported by two wooden supports of approximately 40 cm in length. During the experiment, the temperature was measured with a maximum and minimum thermometer, keeping the average of 25 °C constant.

The endophytic bacteria used in this work was isolated from tomato seeds extracted from fruits produced in the Horticulture sector of UFRRJ in 2008 and received the registration number ENA 4593.

Phenotypic characterization

Phenotypic characterization was performed using API 20 NE Kit (Biomerieux) according to the manufacturer's instructions. The test combines in one database eight conventional enzyme reaction tests (nitrate to nitrite or nitrogen reduction, tryptophan degradation, glucose fermentation, arginine dihydrolase production, urease production, esculin hydrolysis, gelatin hydrolysis and p -nitrophenyl-b-galactopyranidase) with 12 carbon

assimilation tests (D-glucose, arabinose, mannose, mannitol, n-acetyl glucosamine, maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenyl acetic acid). Whether or not these substrates are used by the isolates generates a biochemical profile of the isolate that is compared to the organism profiles of the kit database.

Sequence and analysis of 16S rRNA genes

Genomic DNA was isolated from pure culture. A large fragment of 16S rRNA was amplified by polymerase chain reaction (PCR) using primers 5'-CTA.CGG.CAA.GGC.GAC.GCT.GAC.G-3') (Versalovic, Schneider, De Bruijn & Lupski, 1994). The amplification reaction contained the end volume of 25 µl, containing: sterile Milli-Q water, 13,8 µl; dNTPs, 5 µl (1.5 mmol.L^{-1} stock from each base); 10X buffer (500 Mm KCl; 200 Mm Tris-HCl, pH 8,4), 2,5 µl; 1.5 µL $MgCl_2$ (50 mmol.L^{-1}); primer, 1,0 µl 950 pmol $µl^{-1}$); DNA, 1,0 µl (50 ng.$µl^{-1}$); Taq, 0.2 µl (5 U. $µl^{-1}$). The thermocycling conditions consisted of an initial denaturation at 95°C for 7 min, 35 amplification cycles of 94°C for 1 min (denaturation), 40°C for 1 min (annealling), 65°C for 8 min (extension) and final polymerization at 65°C for 16 min, 4°C. PCR product was purified and sequenced. The 16S rRNA gene sequences generated were assembled using the Phred / Phrap / Consed programs on a Linux operating system (Ewing, Hillier, Wendl & Green, 1998a; Ewing & Green, 1998b; Gordon, Abajian & Green, 1998). From obtaining the sequencing result, homology to the known gene sequence was analyzed through BLASTn against the National Center for Biotechnology Information-NCBI platform database (http: //www.ncbi.nlm.nih. gov).

Inoculum Preparation

The inoculum of ENA 4593 was obtained by removing a 0,1 mL aliquot from the bacterial stock culture kept under refrigeration. For the *in vitro* experiment the bacterial suspension was reactivated metabolically in NA culture medium, sown in triplicates and transferred to a bacteriological greenhouse for growth for 24-48 hours. After bacterial colonies grew, the inoculum concentration was adjusted to the equivalent of 10^8 CFU / mL.

Before undergoing the treatments, the seeds were superficially disinfected with a 50% alcohol solution for 30 seconds, 0.7% sodium hypochlorite for 3 minutes and successive washes of sterile distilled water in a vortex shaker with five water changes.

After disinfestation, the seeds were aseptically transferred to sterile Erlenmeyer flasks containing 40 mL of sterile distilled water (control) and the other containing 40 mL of

bacterial suspension with a final concentration of 10^8 CFU / mL present in the solution (inoculum).

For inoculation the flasks were coupled to a vacuum pump desiccator, subjected to a pressure of 680 mm Hg, for three successive cycles of five minutes, interspersed with three minutes of rest. Immediately after inoculation, the seeds subjected to the treatments were sieved and placed to dry in an UV Laminar Flow Chamber for one hour.

Seed Germination

The disinfected tomato seeds were placed to germinate in previously sterile Gerbox (MYLABOR, NETLAB - BRAZIL) boxes containing germitest paper. To these boxes were also added 8 mL of sterile distilled water, in order to maintain the necessary humidity during the development stage of the plant.

In the growth chamber, the seeds germinated so that the epicotylus turned to the lower position of the box, stimulating the vertical growth of the root. After root protrusion, the seeds were selected according to the pre-established root length for the laboratory tests (8-12 mm).

Experiment I: 7%, 21% Polyethylene glycol (PEG_{6000}) and *Serratia* sp.

For the simulation of osmotic stress, concentrations of 0%, 7% and 21% of PEG_{6000} were used, corresponding to the water potentials of 0.0 MPa, -0.08MPa and -0.54MPa, respectively.

The pre-selected tomato seeds were transferred to sterile 15 cm long test tubes containing 2 mL sterile distilled water plus a 10 x 1.5 cm germitest paper strip soaked with the following solutions: sterile distilled water (control), 7% and 21% PEG solution.

The tubes were closed with hydrophilic cotton, and transferred to the growth chamber with the respective treatments, remaining during the experimentation period. The seeds without inoculum (control) and with inoculum (*Serratia* sp.) were transferred to sterile assays containing germitest paper and 2 mL of sterile distilled water in order to maintain necessary humidity for the germination period. Seed treatments without inoculum (control), with inoculum (bacterial) and different concentrations of PEG were maintained during the experimental period, where later data collections were made. After this period, the seedlings containing the rootlets were removed from the tubes and then measured the variables: root net length, hypocotyl length, cotyledonary length and number of lateral roots, using a digital caliper.

The experimental design used was completely randomized with 8 repetitions for each treatment. The results were statistically interpreted and subjected to analysis of variance (ANOVA) of the data at significance of 5% and 1% probability by the F test with the aid of Microsoft Excel and SAS. Comparison of differences between means was performed by means of the least significant difference (LSD) test at the 5% significance level.

Indoleacetic acid (IAA) production

The analysis of the quantification of auxins produced by the ENA 4593 isolate was performed using the colorimetry technique. This technique was proposed by Rodrigues, Oliveira, Vidal, Simões-Araújo & Baldani (2010), who used for this analysis modified LGI-P culture media, Dygs and C2, as well as tryptophan, synthetic IAA and Salkowski's reagent solutions (Gordon & Weber, 1951). For the construction of the IAA calibration curve increasing concentrations of 0 to 1000 µM/mL of IAA were used according to the method described by Sarwar & Kremer (1995) 150 µl aliquots of each dilution were transferred to polystyrene microplates and then packaged 100 µl of Salkowski reagent. After 30 minutes of reaction in the dark at room temperature, the absorbance was read on a spectrophotometer at a wavelength of 540 nm. The data obtained were processed using Ascent Software for iEMS Reader MF software and plotted as a scatter plot.

The isolated ENA 4593 from *Serratia* sp. it was cultivated in microplates containing modified LGI-P medium, which was subjected to the presence and absence of tryptophan during its cultivation period, characterizing two distinct experiments.

After inoculation, the microplates were incubated at 30°C with shaking until complete 4 days. In the second experiment, however, the isolate was evaluated by the presence of L-tryptophan in colony cells (solid medium LGI-P) and liquid culture (washed cells and adjusted to Optical Density - OD620nm). After 168 hours, the bacterial growth by OD620nm in microplates was evaluated and measured. The cultures were then centrifuged and the supernatant collected for later use in spectrophoorimetric reactions. Auxins concentration analysis was performed by determining the IAA calibration curve.

Experiment II: Different concentrations of IAA

The preparation of the synthetic plant hormone IAA solution (GoldBio.com brand) was made from 175 mg dissolved in 2 drops of 0.5M Potassium hydroxide (KOH) until complete dissolution to complete one liter of water, thus generating the 10^{-3} mol L^{-1} solution.

Following this procedure, serial dilutions in sterile distilled water were made to 10^{-3}, 10^{-6}, 10^{-10} and 10^{-13} mol L^{-1} decimal concentrations.

For this assay, two distinct *in vitro* seed cultivation steps were proposed, maintained in a growth chamber at 25°C, with a 12-hour photoperiod under fluorescent lighting.

In the first stage, an experiment was set up to evaluate the effect of different dosages of phythormonium IAA on tomato, and the second stage was to verify the effect of *Serratia* sp. and different concentrations of IAA in tomatoes. Thus, in the first stage the assay consisted of five treatments: tomato seed in different decimal concentrations of IAA (10^{-3}, 10^{-6}, 10^{-10} and 10^{-13} mol L^{-1}); tomato seed and sterile distilled water (control).

The second stage was also mounted in test tubes containing different types of treatments: control (seed and sterile distilled water), *Serratia* sp. (seed inoculated with strain ENA 4593 and distilled water) and different doses of IAA (seed and decimal concentrations 10^{-3}, 10^{-6}, 10^{-10} and 10^{-13} mol L^{-1}).

For the two distinct stages, eight repetitions were considered for each treatment arranged in a completely randomized way. At the end of two days, data were collected and the variables analyzed along the root segment were: net root length, hypocotyl length, cotyledonary length and number of lateral roots, using a digital caliper.

The different concentrations of IAA were adjusted according to the best equation for the 2nd degree regression coefficient, tested by the t-test corrected for variance analysis residues.

3. Results and Discussion

Phenotypic and molecular characterization

The analysis of the phenotypic characterization of the ENA 4593 isolate from the references described in the Kit (Biomerieux) showed that the isolate presented typical characteristics of the genus *Serratia*. According to Grimont & Grimont (2006), the genus *Serratia* is defined by the ability to assimilate carbon through the fermentation of glucose, mannitol, maltose, malate, mannose, n-acetyl glycosamine, potassium gluconate, citrate and phenyl acetic acid. The authors also reveal that all *Serratia* species have negative results for enzymatic reactions of urease production, arabinose, arginine, tryptophan deaminase degradation and absence of H_2S production.

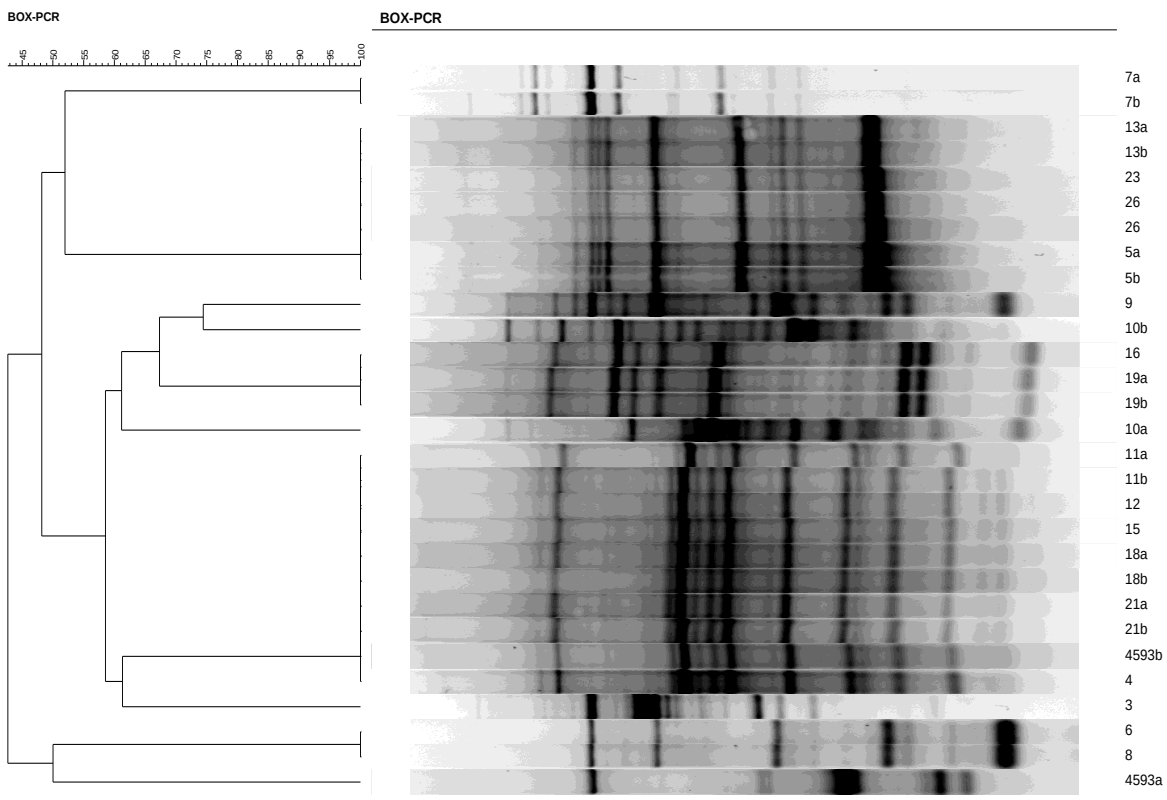


Figure 1: Dendogram of tomato seed endophytic isolates, based on sequencing of 16S rRNA regions, preserved and stored at the Laboratory of Seed Epidemiology and Pathology (LabEPS), UFRRJ. **Source:** BLAST Program

Figure 1 shows the endophytic isolates extracted from Santa Clara tomato seeds, the sequencing made it possible to identify the strain ENA 4593 as belonging to the *Serratia* genus when comparing its phylogenetic profile with other bacteria also isolated from tomato seeds. The molecular characterization from 16S rRNA gene sequencing made it possible to phylogenetically group different strains of microorganisms, revealing high similarity (99% reliability) according to the homology sequence provided by the BLAST Program (Figure 1).

After separation of the genetic profiles of the isolates, one or more representatives of each group were selected for DNA extraction and subsequent sequencing for identification (Table 1).

Table 1: Selection of DNA Extraction Isolate Groups

Profile	**Grouping of genetic profiles**	**Isolate selected for DNA extraction**
1	7a e 7b	7[a]
2	5a, 5b, 13a, 13b, 23, 26	13[a]

3	9	9
4	10b	10 b
5	16, 19a, 19b	16, 19ª
6	4, 11a, 11b, 12, 15, 18a, 18b, 21a, 21b e **4593**	**11a, 21a,**
7	3	3
8	6 e 8	6

Isolate Code: one lowercase letter. **Source:** Authors

The result described in table 1 shows that the isolate ENA 4593 was grouped together with other genetic profile]s (4, 11a, 11b, 12, 15, 18a, 18b, 21a, 21b), strains 11a and 21a being chosen for sequencing, shown in the Table 2.

Table 2: Molecular identification of LabEPS tomato endophytic isolate.

Profile	**Isolates grouped in profile**	**Isolated Selected**	**Selected Molecular Identification**
6	4, 11a, 11b, 12, 15, 18a, 18b, 21a, 21b e 4593 e 21ª	11a, 21ª	*Serratia* sp. *Serratia* sp.

Source: Authors

Molecular sequencing revealed that isolates 11a and 21a were identified as the bacterium *Serratia* sp. (Table 2).

***In vitro* bioassays**

Osmoconditioning promoted a significant effect ($p < 0.001$) on tomato axial root growth when submitted to stress of -0.08 MPa (PEG 7%) and bacterialization by *Serratia* sp. (Table 3). Stress data imposed by -0.54 MPa (PEG 21%) reveal no significant difference in relation to control, but differ significantly from PEG concentration at 7%.

Table 3. *Solanum lycopersicum* L. liquid root mean lengths treated with 7% PEG concentration; 21% PEG and ENA 4593 isolate from *Serratia* sp.

Tratament	Means (mm)	5%
Control	32.583	B
PEG 7%	69.343	A
PEG 21%	34.68	B

Serratia sp.	76.797	A

Means followed by the same letter do not differ significantly ($p < 0.05$) by the LSD test. **Source:** Authors

The interaction unfolding also indicates that tomato seedlings treated with osmotic stress of -0.08 MPa did not differ significantly from seedlings inoculated with *Serratia* sp. (Table 3), suggesting that the tomato tends to grow more when inoculated with the bacteria, thus promoting the modification in root architecture. The similar results from PEG 7% and *Serratia* sp. inoculation indicates that, at least partially, the effect of about this bacteria is physical.

Cardoso et al. (2012) corroborates the data found for PEG 21%, when it reveals that the effect of osmotic stress of -1.0 MPa on root growth of *Myracrodruon urundeuva* fr. *German* (aroeira-do-sertão) did not differ significantly from the control.

Mundada, Nikan, Anil Kumar, Umdale & Ahir (2020) when evaluating the effect morpho-physiological and biochemical responses of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes to PEG-induced osmotic stress, found that in all PEG solutions (5%, 10%, 15%, 20% and 25%) tested, there was a significant decrease in the percentage of germination and growth parameters (root length, shoot length and root/shoot ratio) of millet genotypes, when compared to control. According to the author, the increasing PEG solutions induced greater osmotic stress in the different millet genotypes, however, significant decrease in seed germination percentage was significantly more at 25% PEG_{6000} treatment. Corroborating the data observed by the above authors Pereira, Martins, Souza & Martins (2012) states that the effects of water stress imposed by PEG on plant development can occur at different stages, especially germination.

Based on the data, it is probably suggested that the mechanism associated with increased tomato root growth in 7% PEG treatments is at least part of the physical effect and not just chemical, as has already been shown for the effect of humic acids in studies by Asli & Neumann (2009) indicating that there is a physical effect of humic acids to reduce hydraulic conductivity in the roots.

Another factor supporting the explanation that the root growth promotion observed in tomatoes when treated with 7% PEG is in part a physical effect is the fact that this molecule is a polymer that restricts water mobility in plants, either by osmotic potential reduction, osmotic stress or colloidal stress effect, root pore clogging such as the results described by Asli & Neumann (2009, 2010a) when using nanoparticles and humic acid. It is also believed that such a physical effect can be reproduced by the bacterium *Serratia* sp. when they

colonize the root and form the biofilm, since the biofilm could be hypothetically reducing water ingress to the root, promoting root growth similar to observed in the 7% PEG test.

The results of the quantification of auxins produced by the ENA 4593 isolate is shown in Table 4.

Table 4. Ability of *Serratia* sp. in producing IAA compared with other bacteria from the bacteriology library of the Genetics and Biochemistry-Agrobiology-Seropédica laboratory/RJ.

Strain	**IAA Concentration (µg. mL^{-1})**
Gluconacetobacter diazotrophicus (PAL5)	30.28
Herbaspirillum seropedicae (ZE78)	40.27
Azospirillum brasilense (SP7)	28.01
***Serratia* sp. (a)**	**18.88**
***Serratia* sp. (b)**	**17.29**

Caption: Repeats are represented by the letters a and b. **Source:** Authors

The results obtained regarding the ability of the isolate ENA 4593 from *Serratia* sp. producing IAA, revealed that this microorganism produced low levels of AIA when compared to the microorganisms present in the library of the EMBRAPA-Seropédica Laboratory of Genetics and Biochemistry. While the bacterium *Serratia* sp. produced on average 18.08 µg.mL^{-1} of IAA, the bacterium *Herbaspirillum seropedicae* produces 40.27 µg. mL^{-1} of IAA (Table 4).

The results found by Radwan, Mohamed & Reis (2004) corroborate the data of the present work revealing that the species *Azospirillum lipoferum* produced low indole compounds indices when compared to other strains of the genus *Azospirillum*.

Similar data were also described in the study by Noori & Saud (2012), which evaluated growth promotion through different mechanisms, including the production of IAA by different strains of bacteria of the genus *Pseudomonas*. Revealing that *P. fluorescens* and *P. luteola* strains were able to synthesize IAA at concentrations of 20.5 µg. mL^{-1} and 19.0 µg. mL^{-1}, respectively.

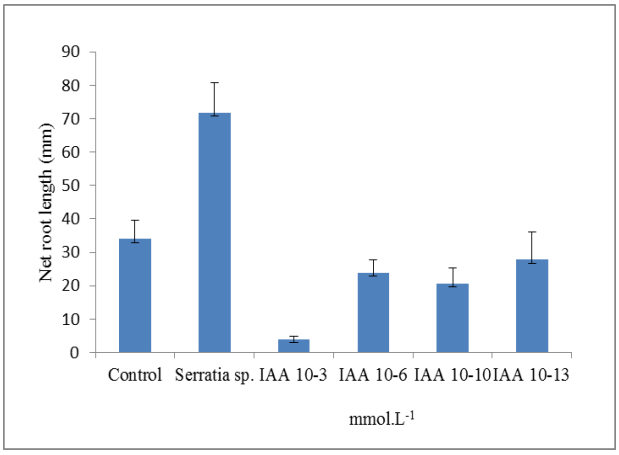
Studies reporting the characteristics of plant growth promoting bacteria associated with the ability to synthesize auxins are many; among them we can mention the works of Dinesh et al. (2015), Ahemad & Kipret (2014), Kavamura et al. (2013). The synthesis of auxin by endophytes is commonly associated with the benefits of promoting axial root

growth. However, the work developed by Schlindwein et al. (2008) with the initial development of lettuce seedlings inoculated with rhizobacteria, revealed that the bacteria that had low IAA production were able to increase the seedling germination and vigor parameters. Bacteria that produced high concentrations of IAA caused an increase in abnormal plant formation and low seed vigor.

According to Tiepo et al. (2018), Lee et al. (2004), IAA is one of the phytohormones capable of regulating and promoting plant development through changes in root architecture, interfering with root size and distribution, which implies a greater absorption capacity of mineral nutrients.

In order to elucidate the growth promoting mechanism exerted by the bacterium *Serratia* sp. in tomato plants bioassays were made comparing different concentrations of IAA. The evaluation of increasing decimal concentrations of IAA on tomato seedling growth was determined from the concentration that would best promote the development of tomato seedlings. These, data described in figure 2, were obtained through bioassays performed during July 2015.

A



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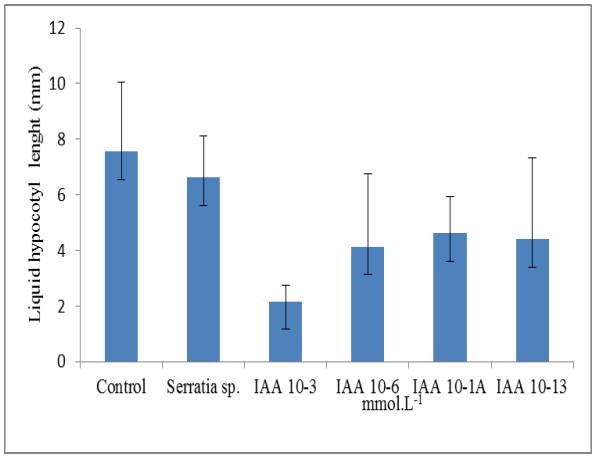

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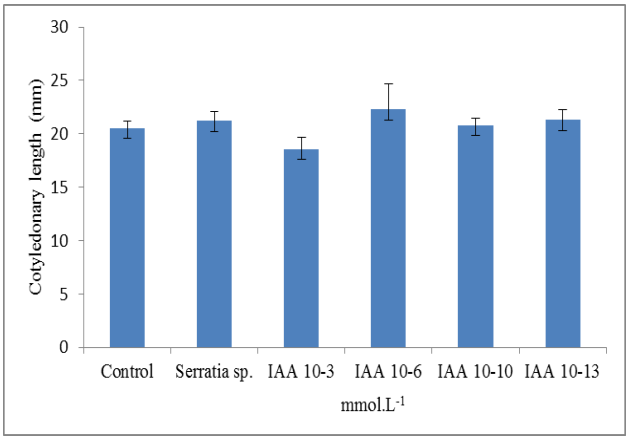

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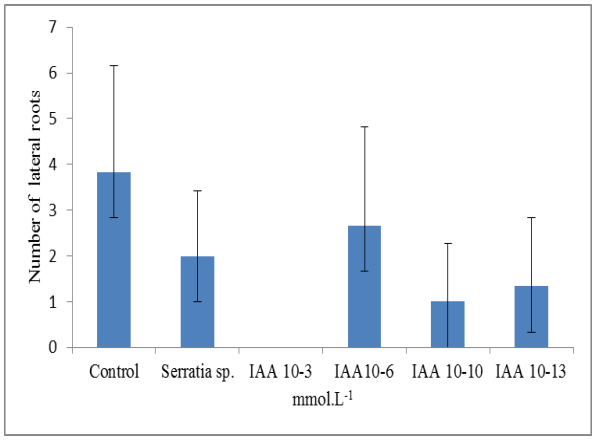

Figure 2: Mean and standard deviation (X ± SD) of the variables analyzed during the evaluation of the effect of *Serratia* sp. and different concentrations of IAA. Net root length (A), Net hypocotyl length (B), Cotyledonary length (C) and Number of lateral roots, performed during January 2015. **Source:** Authors

According to Tabatabaei (2016) the response of the plant to IAA produced by bacteria can have both beneficial and depressing effects. The IAA-derived ethylene is believed to participate in the disruption of the normal growth (i.e. germination and seedling growth) of the host plant. The data found in the present study (Figure 2) are in line with the results described by the author above who, when testing different concentrations of exogenous IAA, in durum wheat seeds, found that high concentrations of this phytohormone, induced inhibitory effects on germination and seedling vigor.

Regression analysis of root net length during the imposition of different concentrations of IAA showed a significant linear trend and an R^2 = 0.802, revealing that higher concentrations of IAA promoted lower growth of the main root (Figure 3). These results suggest that the promotion of axial root growth observed in tomato could not be associated with auxin. Since, the highest concentration of IAA (10^{-3} mmol L^{-1}) was the one that most reduced net root growth.

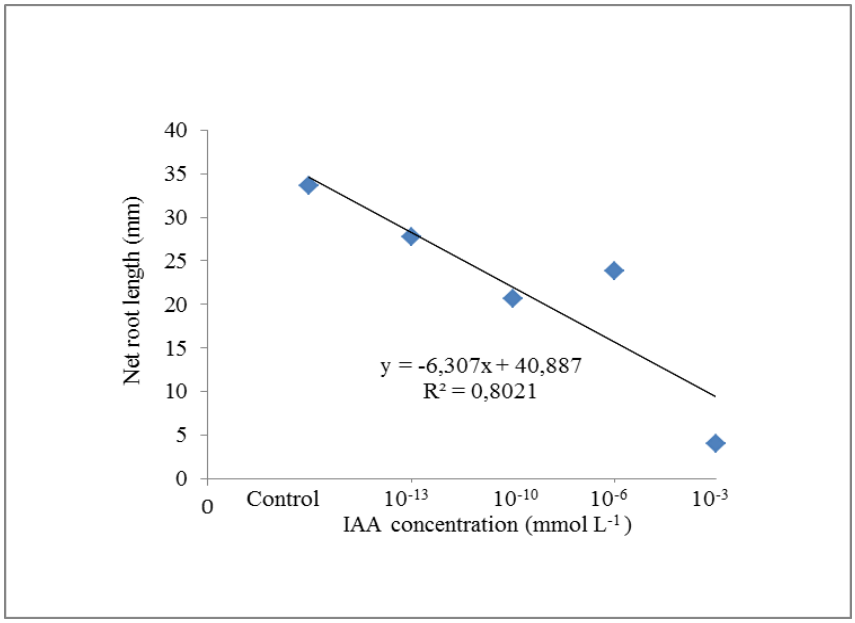

Figure 3: Net root length of tomato seedlings submitted to different concentrations of IAA (mmol L^{-1}). **Source:** Authors

The negative IAA effect observed on the root growth (Figure 3) reinforces the hypothesis that the mechanism of growth promotion induced by the bacterium *Serratia* sp. is partly associated with the same physical effect seen with 7% PEG treatment.

The work described by Braz et al. (2010) corroborates the data found in this work since when testing auxin and humic acid concentrations in the Arabidopsis thaliana plant, it was found that high concentrations of IAA induced a linear reduction in root growth when compared to control plants, in contrast, the effect of HA tended to induce the increase of this variable in all concentrations studied, with a significant effect on the concentration of 40 mg L^{-1}. Also, according to the same authors HA modified the morphogenesis of the roots of the plant, promoting the increase of the main root, as well as the number of lateral roots, suggesting that the acting mechanism are associated with the bioactivity of HA.

The results found by the aforementioned authors are in agreement with the results also described in the study by Dobbss et al. (2007) reinforcing the hypothesis that the mechanism associated with the growth promotion of *Serratia* sp. bacteria can be explained by a physical effect of HA and PEG, but cannot be reproduced by auxin.

The data found in this paper suggest a new discussion around the effect of auxins on plant growth promotion mediated by rhizobacteria, as many authors, such as Ahemad & Kibret (2014), have been known to highlight the benefits of plant growth through the synthesis of phytohormones. Another factor that corroborates the results found is the low levels of IAA produced by the bacterium *Serratia* sp., suggesting that the mechanism of growth promotion induced by this endophyte would not be associated with the synthesis of this hormone.

Taken together, the results from the present work indicate that the effect of *Serratia* sp. on root growth is physical, as PEG, and not hormonal, as IAA.

4. Final considerations

Further works should investigate whether the knockout of the auxin synthesis genes in *Serratia* sp. affect its ability to enhance root growth. Another investigation could also be the use of *Serratia* sp. in field experiments with tomato under drought, to evaluate its putative benefits helping plant to cope this important stress.

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