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Article

Comparison of organically derived IAA and synthetic IAA in rosa Forever cuttings

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Abstract

The genus *Rosa* sp. Forever Young, belonging to the rose family, is the most important crop in the ornamental sector, which represents one of the main products in the floriculture market. The propagation of roses is usually asexual and is done by cuttings or grafts. Auxins, such as indole acetic acid, stimulate root formation and increase the percentage of rooting of cuttings and survival, so they are used to improve propagation. The research aimed to determine the effect on vegetative propagation of cuttings of *Rosa* sp. Forever Young with synthetic indole acetic acid and with organically derived indole acetic acid present in supernatants from culture of the strain of *Pseudomonas guariconensis* RMC6. Cuttings of approximately 10 cm were obtained and immersed for 3 h in each treatment. The research was conducted in a completely randomized experimental design, with eight treatments, with four replications. The experimental unit was a pot with 25 cuttings per pot, having a total of 100 cuttings. It was observed that the interaction given by the inoculation between the cuttings of rosa Forever and the organically derived indole acetic acid present in the culture supernatants of the strain had a positive effect on the variables of root length, root diameter, root number, stem diameter, and number of shoots; the replacement of synthetic auxin with the organically derived auxin from the strain of *P. guariconensis* RMC6 can be suggested.

Keywords:

Pseudomona guariconensis, auxins, vegetative propagation, strain.

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Introduction

The genus *Rosa* sp. Forever Young, belonging to the rose family, is the most important crop in the ornamental sector, which represents one of the main products in the floriculture market (Fernández *et al*., 2014); its importance is such that it is estimated that it participates with 25% of the national production in ornamentals (SIAP, 2020).

The propagation of the *Rosa* sp. Forever Young is usually done by cutting or grafting (Sitinjak, 2015). To be successful in the attachment of the vegetative parts of the plants of these plants, rooting compounds are used, which help the proliferation and formation of a good root system that allows the growth and development of a new plant (Quispe, 2017).

Auxins, such as indole-3-acetic acid (IAA), stimulate the formation of adventitious roots and increase the percentage of rooting of cuttings and survival; the amounts used are very small because they can cause deforming effects on the tissue or inhibit growth (Grossmann, 2010; Kashefi *et al*., 2014; Márquez *et al*., 2018). Auxin indole-3-acetic acid is a phytohormone of great importance for plant development, which can be synthesized through various metabolic pathways, such as through IAA-producing bacteria (Vega-Celedón *et al*., 2016).

There are some bacteria that favor root development, producing IAA (Leveau and Lindow, 2005). Tryptophan is the main precursor in IAA biosynthesis pathways in bacteria (Patten and Glick, 2002; Spaepen *et al*., 2007; Tsavkelova *et al*., 2007). Felker *et al*. (2005) conducted a study with cuttings of *Prosopis alba*, inoculating them with *Pseudomonas aurantiaca*, obtaining an 80% rooting in the cuttings. Likewise, Kaymak *et al*. (2008) inoculated cuttings of *Mentha piperita* with different strains and reported that they obtained greater root length in cuttings that were inoculated with the strains compared to the control that was not exposed to bacterial suspensions.

Producers have chosen to produce seedlings themselves; their problem is to choose the best way to propagate roses since, by employing a specific method, there is no guarantee that their product will be of quality and even economically profitable (Ulcuango, 2019). In addition to this, flower companies find themselves in need of a better quality product without affecting the environment and contaminating the soil, being more competitive by increasing production, minimizing costs, and maximizing profits.

For this reason, new management practices are sought, such as the use of biological products that offer an alternative, in a responsible way to improve agricultural production (De la Cadena-Vera, 2005). The research aimed to evaluate three different concentrations of synthetic IAA of reagent grade and organically derived IAA from the strain of *P. guariconensis* RMC6 in cuttings of *Rosa* sp. Forever Young.

Materials and methods

The research was conducted in a greenhouse in the experimental field of the Faculty of Agricultural Sciences-Autonomous University of the State of Morelos (UAEM), for its acronym in Spanish, with a geographical location of 18° 58’ 54.71” north latitude and 99° 13’ 59.14” west longitude, and 1876 masl (García-Rubio *et al*., 2015).

Plant material

Mother plants of *Rosa* sp. Forever Young in an 8” pot were acquired from a marketing company in Cuautla, Morelos. Homogeneous stems were chosen and cuttings of approximately 10 cm were obtained, taking care that the cuttings had at least two nodes with their respective buds; the basal part was cut in a bevel shape.

Culture medium

The strain used was the strain of *P. guariconensis* RMC6 isolated from the rhizosphere of a corn plant grown in the Molecular Pathogenicity Laboratory of the Center for Research in Cell Dynamics

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of the Autonomous University of the State of Mexico (UAEM), for its acronym in Spanish, which had previously been identified and evaluated, determining that the *P. guariconensis* strain promoted plant growth and synthesized IAA using the precursor tryptophan and it release it into the culture medium. The nutritious broth (Bioxon) was prepared by dissolving 8 g of the dehydrated medium in 1 L of distilled water. The suspension was heated to 55 °C until it was completely dissolved. It was then placed in bottles of culture medium and sterilized at 120 °C for 20 min.

Preparation of organically derived IAA from the strain of *P. guariconensis* RMC6

The bacterium was cultured in the nutrient broth (Bioxon) in the presence of tryptophan (0.5 g L^{-1}) (Luziatelli *et al*., 2020) for 24 h at 30 °C in stirring (150 rpm). The bacterial concentration was adjusted to 10^8 cells ml^{-1}. Subsequently, to obtain the bacteria-free culture supernatant, the cells were centrifuged at 10 000 rpm at 4 °C for 10 min to separate and remove the cell pellet.

The culture supernatant was separated by decantation and sterilized by filtration using polyethersulfone (PES) filters with a pore size of 0.22 µm. To quantify the amount of IAA produced, an aliquot of 400 µl was taken and vigorously mixed with 1 600 µl of Salkowsky reagent [150 ml of sulfuric acid (H_2SO_4), 250 ml of distilled water (H_2O), 7.5 ml of iron chloride ($FeCl_3$) 0.5 M]. Samples were incubated at 28 °C in darkness for 30 min and absorbance was measured at 530 nm.

A standard curve was made with IAA to determine the concentration; the amount of organically derived IAA from the strain of *P. guariconensis* RMC6 was expressed in µg ml^{-1} (Cordero *et al*., 2014). The culture supernatant was diluted with sterile nutrient broth to adjust the desired concentrations (17 µg, 8 µg, and 5 µg IAA ml^{-1}).

Preparation of reagent grade synthetic IAA

The synthetic IAA was prepared at 10 mg ml^{-1} of sterile water (Khan *et al*., 2006); from this solution, the desired concentrations of 17 µg, 8 µg, and 5 µg were prepared to match the concentrations of the culture supernatant of the strain. Eight treatments were established for the evaluation of organic IAA from the strain of *P. guariconensis* RMC6 and synthetic IAA (Table 1).

Table 1. Description of the treatments evaluated for rooting cuttings of *Rosa* sp. Forever Young.

Num. of treatment	Treatment
T1	Control-cuttings with water alone
T2	Control with culture medium of (nutrient broth, Bioxon)
T3	Organically derived IAA from the strain of *P. guariconensis* RMC6, at a concentration of 17 µg with culture medium (nutrient broth, Bioxon)
T4	Organically derived IAA from the strain of *P. guariconensis* RMC6, at a concentration of 8 µg with culture medium (nutrient broth, Bioxon)
T5	Organically derived IAA from the strain of *P. guariconensis* RMC6, at a concentration of 5 µg with culture medium (nutrient broth, Bioxon)
T6	Sigma® synthetic IAA of reagent grade, at 17 µg with culture medium (nutrient broth, Bioxon)
T7	Reagent grade synthetic IAA, at 8 µg with culture medium (nutrient broth, Bioxon)
T8	Reagent grade synthetic IAA, at 5 µg with culture medium (nutrient broth, Bioxon)

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Application of treatments

For T1, the cuttings were left immersed in 60 ml of distilled water for 3 h to resemble the conditions of the organically derived IAA from the strain of *P. guariconensis* RMC6 and the synthetic IAA. For T 2, the cuttings were left submerged in 60 ml with culture medium for 3 h.

To inoculate the cuttings in treatments T3, T4 and T5 with organically derived IAA from the strain of *P. guariconensis* RMC6, the cuttings were placed by the basal part and immersed in 60 ml of the culture supernatant of the bacterial strain for 3 h, as reported by Tariq *et al*. (2016).

The treatments T6, T7 and T8 from synthetic IAA were immersed by the basal part for 3 h in 60 ml with culture medium to equalize the conditions. After this time, in all treatments, the cuttings were planted in 6” pots that were previously filled with Sunshine Mix® commercial substrate. Once the cuttings were established, they were irrigated every third day. The application of Foligreen 18-18-18 was carried out every nine days, replacing irrigation until the end of the experiment.

Experimental design

The research was carried out in a completely randomized experimental design, with eight treatments (Table 1), with four replications. The experimental unit was a pot with 25 cuttings per pot, having a total of 100 cuttings.

Study variable

Ninety days after establishing the cuttings, the following response variables were evaluated: percentage of survival (%), root length (mm), measured considering the longest root of the cutting, root diameter (mm), measuring the middle part of the longest root of the cutting, seedling length (mm), from the basal part to the apical part of the stem, and stem diameter (mm), measured in the middle part of the stem. The number of roots and shoots per cutting were also counted.

Data analysis

Data were analyzed using analysis of variance (Proc Anova, $p\leq 0.05$) and the comparison of means test (Tukey, $p\leq 0.05$) with the SAS 9.0 statistical package (SAS, 2002).

Results and discussion

The analysis of variance shows highly significant differences (p# 0.05) between the treatments with respect to the study variables of root length, root diameter, stem length, stem diameter, number of shoots, and fresh root weight, and it was significant only for the number of roots (Table 2).

Table 2. Mean squares (MS) and coefficient of variation (CV) of the analysis of variance of six variables evaluated in eight treatments in *Rosa* sp. Forever Young.

Variable	MS	CV (%)
(%) of survival	105.5 *	10.99
Root length (mm)	667.6 **	11.73
Root diameter (mm)	15.61 **	29.95
Num. of roots	2.18 *	12.5
Seedling length (mm)	622.8 **	7.9
Stem diameter (mm)	12.03 **	22.32
Num. of shoots	0.609 **	14.1

** = highly significant $p\leq 0.05$; * = significant $p\leq 0.05$.

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The results obtained in the survival percentage showed that the highest percentage of live cuttings was in T6 (84%), which was synthetic IAA of reagent grade at a concentration of 17 µg with culture medium, followed by T4 (79%), which was constituted by organic IAA from the strain of *P. guariconensis* RMC6 at a concentration of 8 µg with culture medium, and by T5 (78%), also constituted by organic IAA from the RMC6 strain at a concentration of 5 µg with culture medium. As expected, the worst treatment, where only 25% survival was obtained, was T1 with just water (Figure 1 and Table 3).

Figure 1. Survival percentage at 90 days after inoculating the cuttings of *Rosa* sp. Forever Young in eight different treatments. The values are expressed as mean ± standard error (n= 100).

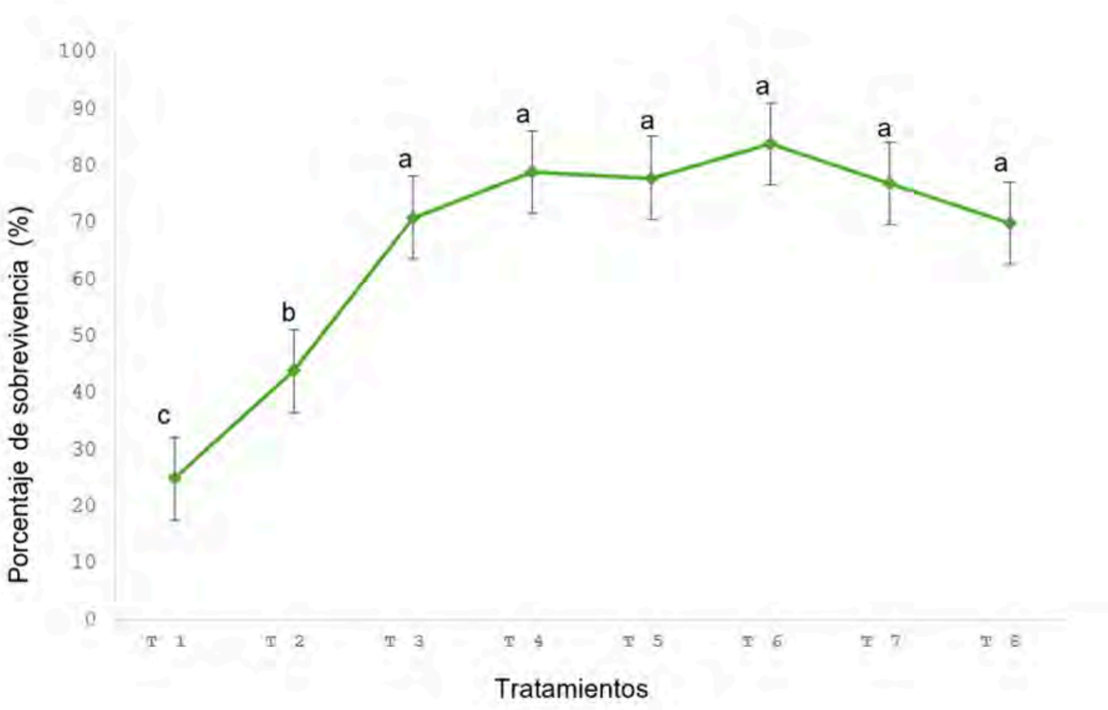

Table 3. Mean values of eight treatments applied to *Rosa* sp. Forever Young.

Treatments	PS (%)	RL	RD	NR	SL	SD	NS
T1	25 c	52.5 c	1.75 b	6 ab	78.5 c	2.1 d	0.67 c
T2	44 b	6.15 bc	7.27 a	5.1 b	77.15 c	2.65 cd	1.42 b
T3	71 a	54.5 c	3.62 ab	6.37 ab	89.87 ab	7 a	1.5 ab
T4	79 a	84.75 a	6.92 a	7.25 a	91.75 ab	4.57 bc	1.95 a
T5	78 a	64.47 bc	6.42 a	6.05 ab	76 c	2.12 d	1.55 ab
T6	84 a	75.7 ab	7.02 a	7 a	104.2 ab	4.3 bc	1.55 ab
T7	77 a	83.85 a	6.1 a	6.67 ab	109.77 a	5.32 ab	1.82 ab
T8	70 a	79.5 ab	6.7 a	7.25 a	91.75 bc	5.05 ab	1.77 ab
Overall mean	66	69.68	5.72	6.4	89.79	4.14	1.53
MSD ($p \leq 0.05$)	4.24	19.14	4.01	1.89	16.16	2.16	0.5

PS= percentage of survival; RL= root length; RD= root diameter; NR= number of roots; SL= seedling length; SD= stem diameter; NS= number of shoots; MSD= minimum significant difference.

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In their study, Felker *et al*. (2005) inoculated cuttings of *Prosopis alba* with *Pseudomonas aurantiaca* and obtained a rooting of 80% compared to the control. González-Candia *et al*. (2016) isolated 32 bacterial strains and inoculated them in mini-cuttings in *Eucalyptus*, reaching a maximum rooting of 75% compared to the control with 28%.

The results in terms of root length were highly significant (*p*# 0.05), which indicated that the best results were obtained in the cuttings of the treatment T4 (84.7 mm) of IAA from the strain of *P. guariconensis* RMC6 at a concentration of 8 µg with culture medium and in the treatment T7 (83.5 mm) with the reagent grade IAA at 8 µg with culture medium, these being the two best treatments with respect to the means since they were statistically equal, and one of the treatments that presented the shortest root length was the treatment T1 (52.5 mm), which was the control, where they were inoculated with just water (Table 3).

A similar situation was found by Kaymak *et al*. (2008), who inoculated *Mentha piperita* cuttings with different strains and reported that the root length (85.19 mm) of the mint cuttings treated with the strains showed higher values compared to the root length (69.85 mm) of the cuttings of the established controls where the cuttings were not exposed to bacterial suspensions.

Montero-Calasanz *et al*. (2013) also studied the effect of the suspension of the bacterial strain *Pantoea* sp. on the elongation of rapeseed (*Brassica napus*) roots, obtaining that bacterial treatments increased the total length of the roots by three to five times more compared to the control where they only used water.

When evaluating the effect of the treatments T2 (3.3 mm), T4 (6.9 mm), T5 (6.4 mm), T6 (7 mm), T7 (6.1 mm) and T8 (6.7 mm) on root diameter, it was observed that there were no statistically significant differences regarding the use of the strain of *P. guariconensis* RMC6 or synthetic IAA, so it is irrelevant to use one or the other (Table 3).

Regarding the variable of number of roots, which is an important and determining variable in the research since it was desired to determine if the IAA from the RMC6 strain at any of the applied concentration produces the same number of roots as the synthetic IAA of reagent grade. It was observed that there were no statistically significant differences in the treatments T4 (7.3), T6 (7) and T8 (7.3).

The number of roots they generated was similar, so it is observed that the same effect and result is obtained using organically derived IAA and synthetic IAA of reagent grade (Figures 2 and 3), and the treatment where the lowest number of roots was obtained was the treatment T1 (1.8 mm), where there was less attachment of the cuttings, which led to death, because they did not have the ability to absorb nutrients through the root.

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Figure 2. Effect on the number of roots in cuttings of *Rosa* sp. Forever Young, in eight different treatments. The values are expressed as mean ± standard error (n= 100).

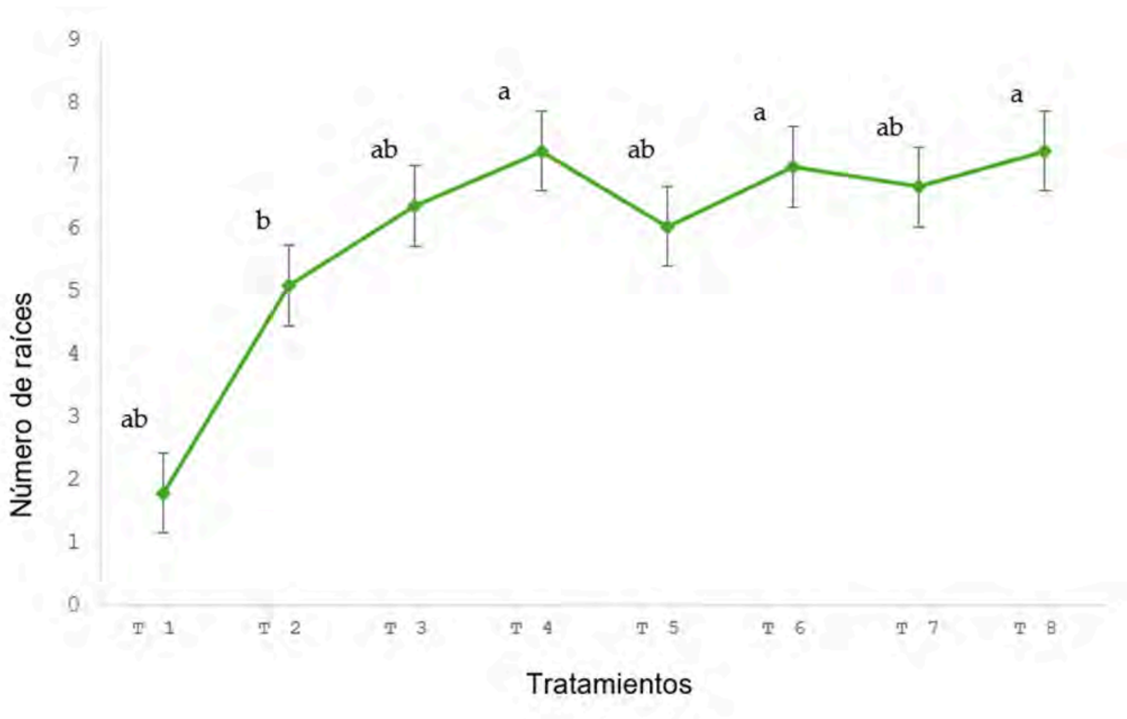
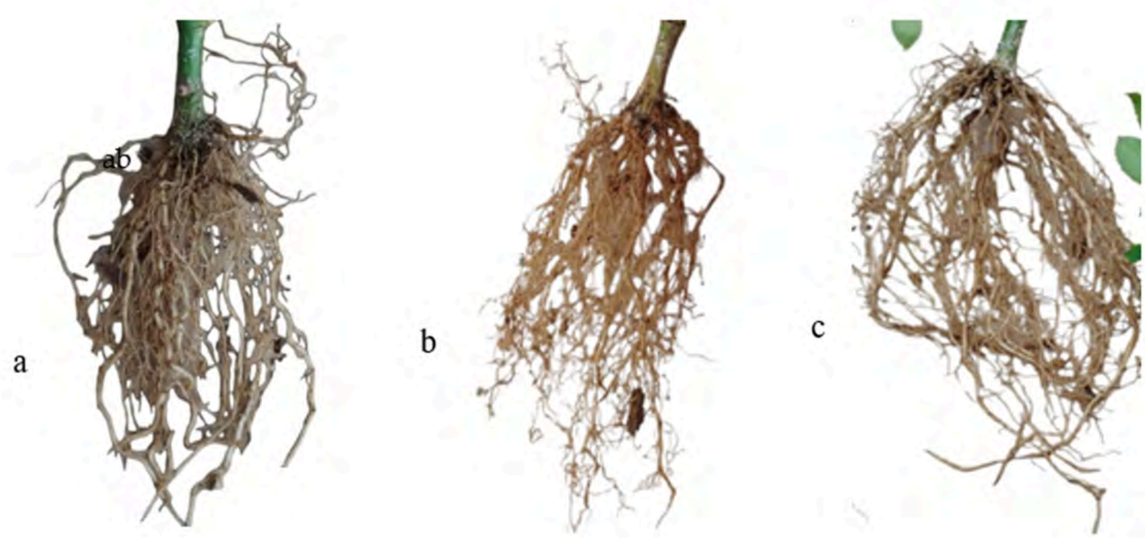


Figure 3. Root number. a) T4: organically derived IAA from the strain of *P. guariconensis* RMC6 at a concentration of 8 µg with culture medium (nutrient broth, Bioxon); b) T6: Sigma ® IAA of reagent grade at 17 µg with culture medium (nutrient broth, Bioxon) and c) T8: Sigma ® IAA of reagent grade at 5 µg with culture medium (nutrient broth, Bioxon).



T2 was not able to induce a greater number of roots (5.1) with culture medium alone since the roots lacked treatment of organically derived IAA from the strain of *P. guariconensis* RMC6 and synthetic

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IAA (Table 3). Díaz *et al*. (2009) conducted a study in eucalyptus cuttings, where the cuttings were inoculated with strains and once established, they were irrigated, as a result they obtained that the studied strains significantly increased the rooting of the cutting and stimulated the development of fine roots, which increased the biomass of the roots compared to the control that was water alone.

In relation to stem length, we observed that the cuttings of T7 (109.8 mm) immersed in synthetic IAA of reagent grade inoculated with 8 µg with culture medium were the ones that showed a highly significant effect on stem length, followed by T6 (104.2 mm) with the cuttings inoculated in reagent grade IAA at 17 µg; these two treatments showed longer length compared to the other treatments that showed different statistical differences among them (Table 3).

From the above, it can be said that the reagent grade IAA stimulates cell elongation in a greater percentage, which is reflected in the growth of the cuttings. Regarding stem diameter, the treatment that showed the highest statistical results was T3, with cuttings immersed in the culture supernatant of the strain of *P. guariconensis* RMC6 that produces IAA, at a concentration of 17 µg with culture medium (Table 3).

It is worth mentioning that, although it was not the purpose of the research, it was observed that the stems of T3 had a more intense green color than the other treatments, so we can infer that this treatment favors chlorophyll in plants. There were no statistically significant differences in the number of shoots in the treatments T3, T5, T6, T7 and T8. The statistically highest was T4, with IAA from the strain of *P. guariconensis* RMC6, at a concentration of 8 µg with culture medium (Table 3).

In this regard, Kamilova *et al*. (2006) inoculated cucumber seeds with *P. fluorescens* WCS365, which generates auxins, and obtained positive results in the increase of the root and in the number of shoots, as observed in the present research where we verified that the IAA from the strain of *P. guariconensis* RMC6 induces the generation of a greater number of shoots, even more than the chemical grade IAA, which is an excellent organic alternative for shoot formation in rose cuttings.

Sezen *et al*.'s (2014) study shows similar results to those of this research, where *Ficus benjamina* cuttings were inoculated with bacterial strains and the result was positive in root length, number of shoots, and number of leaves. Kaymak *et al*. (2008) mention that producers stimulate rooting through the use of growth regulators, including auxins, which play an important role in root development; however, intense use of these could lead to environmental problems.

The irrational use of chemicals has aroused the interest of sustainable and environmentally friendly research (Salantur *et al*., 2005); the inoculation of the strain of *P. guariconensis* RMC6 or culture supernatants can be an excellent biological alternative to promote the rooting of rose cuttings and thus contribute to environmental problems.

It is also important to note that, although it was not the objective of the study, it was observed that during the development of the research, there were pests in the crop (aphids) and diseases (sooty mold); nevertheless, it is worth mentioning that the cuttings submerged with the culture supernatant from the RMC6 strain (T3 and T4) did not present pest or disease problems.

This phenomenon is known as induced systemic resistance (ISR) and focuses on the induction of defense mechanisms promoted by strains of *Pseudomonas* and *Bacillus* (Kloepper *et al*., 2004; Bordiec *et al*., 2011). These defense mechanisms allow restricting or blocking the ability of pathogenic microorganisms to cause diseases, which are regulated through a network of physical interconnections and chemical signaling pathways involving jasmonic acid (AJ) and ethylene (ET), resulting in partial or complete resistance against subsequent pathogen attack (Sánchez and Guerra 2022).

Conclusions

The interaction given by the inoculation between the cuttings of rosa Forever and the IAA present in the culture supernatants of the strain of *Pseudomonas guariconensis* RMC6 showed that there was a positive effect on the variables of root length, root diameter, root number, stem diameter, and number of shoots, this is specifically with the treatment T4, which was composed of organically

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derived IAA from the strain of *P. guariconensis* RMC6 at a concentration of 8 µg with culture medium (nutrient broth, Bioxon), compared to synthetic IAA, which showed equal values in some variables and lower values than treatment T4 in other variables, thus suggesting the replacement of synthetic auxin (IAA) with organic auxin from the strain of *P. guariconensis* RMC6, at least in rosa Forever cuttings where inoculation with the strain of *P. guariconensis* RMC6 can be an excellent organic alternative to promote the rooting of rose cuttings and thus contribute to reducing environmental problems.

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