



Effect of *Rhizobium* Inoculation on Tomato (*Solanum lycopersicum* L.) Yield in Protected Crops [†]

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Abstract: The insufficient availability of nutrients in the soil and the non-use of biofertilizers as a strategy in the tomato nutrition process are factors that limit the yield of this crop. The objective of this research was to evaluate the effect of different *Rhizobium* strains on the yield of the Aegean hybrid tomato variety. The inoculation of the microorganisms was carried out at the time of sowing and transplantation, in a proportion of 10% with respect to the volume of the root ball. The experimental design was in randomized blocks, with four treatments and with four replications for each treatment: an uninoculated control and three levels of the inoculation factor with the strains of *Rhizobium*, *Rhizobium etli* CE-3, *Rhizobium leguminosarum* SCR; *Rhizobium leguminosarum* Semia-4088. The sampling was carried out in a zig zag pattern throughout the field and the following variables were evaluated: dry mass by plant organs, foliar NPK, growth indicators, productive indicators, crop yield, and economic evaluation. The results achieved showed a positive effect on the indicators evaluated in the plants inoculated with the *Rhizobium* strains with respect to the control without inoculation. With the inoculation of the *Rhizobium etli* CE-3 strain, the best results were obtained regarding tomato yield.

Keywords: tomato; inoculation; *Rhizobium*; symbiosis; yield



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1. Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most important and most demanded horticultural crops in the world due to its excellent nutritional properties and its role in the eating habits of a large part of the population, both for fresh consumption and industrial processing; however, its performance is limited by different factors, the most important being the inadequate use of chemical fertilizers and the insufficient availability of nutrients in the soil, mainly nitrogen, affecting the growth and production of this crop.

Among the factors that influence the decrease in tomato crop production, the inappropriate use of chemical fertilizers is the one that most affects the growing deterioration of biodiversity and the microbial balance of the soil, whose damages are sometimes observed in the long term [1]. For this reason, it is currently necessary, as one of the most valuable elements to consider, to promote sustainable agriculture from the use of biofertilizers, which allows for the reduction of the use of chemical fertilizers and improve the absorption and availability of nutrients in the soil. In this sense, it is essential to develop research that promotes new production systems that increase yields and generate excellent quality products, guaranteeing agricultural development without contaminating the ecosystem, while preserving soil fertility and biodiversity.

Various microorganisms reside within the rhizosphere that have the ability to promote the growth of crops of interest, favor the supply of nutrients to the soil or plants, and can be exploited as a sustainable strategy to increase productivity. Within these microbial groups, plant-growth-promoting bacteria (PGPR) stand out, as they act in a coordinated manner at the soil-root interface; this group of bacteria includes the genus *Rhizobium*, which has been widely studied in recent years, such as to check if nitrogen fixation is feasible in non-legume

plants [2]. Among the described biochemical mechanisms exerted by (PGPR) that have beneficial effects on plants is the biological fixation of atmospheric nitrogen (FBN), carried out by symbiotic rhizobacteria such as *Rhizobium* sp. or other free-living species, such as *Azotobacter* sp. and *Azospirillum* sp., that have been used extensively as biofertilizers to improve the availability of nitrogen in vegetables such as tomato (*Solanum lycopersicum* L.), onion (*Allium cepa* L.), and lettuce (*Lactuca sativa* L.) [3–5]. Other mechanisms that promote plant growth include the solubilization of phosphorus (P), as well as the synthesis of phytohormones, vitamins, and enzymes, which reduce the incidence of diseases and pathogens and provide greater tolerance to abiotic stress, as well as the increased absorption of water and nutrients [2,6].

In Cuba, greenhouse tomato cultivation occupies the largest cultivated area, which allows the crop to be protected from adverse conditions such as high temperatures, light intensity, rain, wind, and insects, with the aim of obtaining harvests during non-optimal periods for this vegetable [7]. In 2018, tomato production in Cuba was 43,405 t, while in the province of Santiago de Cuba, a production of 365.3 t was reported [8,9]. In the Protected Crop Unit “Campo Antena” in the same year, the average yield of the Aegean hybrid variety was 60 t/ha.

In the “Campo Antena” unit belonging to the Empresa Integral Agropecuaria Santiago de Cuba, prior to the investigation, chemical analysis evaluations were carried out on the soil, where the insufficient availability of nutrients was determined, causing a decrease in the productivity of the tomato crop. As such, the objective of this research is to evaluate the effect of different *Rhizobium* strains on the yield of tomato (*Solanum lycopersicum* L.).

2. Materials and Methods

2.1. Location and Conditions of the Experiment

The research was development in the Protected Crop Unit “Campo Antena”, coordinates X: 60,7547.321; Y: 15,6420.837, belonging to the Empresa Integral Agropecuaria Santiago de Cuba, from November 2018 to April 2019 on brown soil without carbonates [10]. The chemical and microbiological analyses shown in Table 1 were performed in the Laboratory of Soils, Plants and Waters, of the Department of Biofertilizers and Plant Nutrition, of the National Institute of Agricultural Sciences (INCA).

Table 1. Chemical and microbiological characteristics of the arable soil layer (0–20 cm deep).

pH en (H ₂ O)	MO (%)	P (mg Kg ^{−1})	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
				(cmol _c Kg ^{−1})		
7.25	2.69	182.7	1.03	2.09	26.5	13.2
Microbiological analysis of the soil.						
No. native rhizobia: 1.8 × 10 ⁵ UFC g ^{−1}						

Chemical determinations: pH in H₂O determined by the potentiometer method: soil/solution ratio of 1:2.5; MO (organic matter) Walkley and Black P: 0.1 N H₂SO₄ solution with soil-solution ratio 1:2.5, NH₄Ac cations at pH 7 [11].

2.2. Description of the Experimental Design, Experimental Area, and Applied Treatments

During the investigation, a completely randomized experimental design was used, with an experiment with four treatments, each having four replications. Four houses of protected cultivation of 0.08 ha^{−1} were used for a total experimental area of 0.32 ha^{−1}. These houses had analogous conditions for the experiment. The number of tomato beds per house is 10 and the number of rows per tomato beds is one; the planting frame dimensions were 1.04 m × 0.40 m, and the number of plants per house was 1923. The crop under study was the tomato Hybrid Aegean variety. The stage to be evaluated was from transplantation to final production. The treatments applied in the protected cultivation houses were: (T1)

control without inoculation, and three inoculation factors with the strains (T2) *Rhizobium etli* CE-3, (T3) *Rhizobium leguminosarum* SCR, and (T4) *Rhizobium leguminosarum* Semia—4088.

2.3. Selection of *Rhizobium* Strains and Method of Inoculation at the Time of Transplantation

Before selecting the strains, the native rhizobia colony forming units (CFU mL⁻¹) were counted. This sampling was carried out in a zig zag manner in the four cultivation houses where the experiment was developed to determine the number (CFU) of rhizobia. It was performed by serial dilutions of 1 g of soil in 9 mL of sterile distilled water, which was seeded on Petri dishes with Mannitol Yeast Agar medium, and incubated at 30 °C for 7 days [12]. The strains used are from the stock of the Microbiology laboratory, Department of Plant Physiology and Biochemistry of INCA, from which certified inoculates were obtained in medium, with a concentration of 10⁸ CFU mL⁻¹. At the time of sowing, a 200 mL dose of each *Rhizobium* strain was applied for every 50 kg of seeds as recommended by INCA, with further adaption of the dose to the crop at the time of transplantation, where 30-day sowing positions were used with a mean height of 12 cm, 3 pairs of true leaves, and a thickness of the stem of 4.2 mm. The strains were used in relation to the volume of the root ball, applying a proportion of 10% of the covering of the root balls in each treatment. The day after transplantation, a light irrigation of 0.5 L per plant was applied with acidified water at a dosage of 136 mL of H₃PO₄ at 85%, and 40 g of Premium Chelate per m³ of irrigation water. The management of the plantation was carried out taking into account the technology of the crop and the biotic and abiotic conditions in which they were developed.

2.4. Variables Evaluated

2.4.1. Variables of the Growth and Development of the Plant

These evaluations were made in 10 plants per replicate for a total of 40 per treatment; the evaluations were made 25, 50, and 75 days after the definitive transplant (d.a.t.). The height measurements were made from the base to the last leaf sprout at the apex of the main stem, with the help of a tape measure (Hunter brand 3 m 10 ft × 16 mm) and for the diameter of the stem they were made at its base, with the help of a caliper, (Mitutoyo brand 530–114–200 mm).

2.4.2. Dry Mass by Plant Organs (g Plant⁻¹) and Foliar NPK

These variables were determined in the harvest phase of the third cluster to harvest of the third to last cluster. Five plants were taken for each treatment, and each organ was weighed separately on a Sartorius digital balance BSA 124S Max 120 g. They were dried in a Boxun BGZ oven at 70 °C for 48 h, and each sample was weighed at an interval of 2 h until reaching a constant mass, thereby determining the dry mass of each one by difference. The foliar NPK analysis was carried out in the Soil, Plant and Water Laboratory of the Department of Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences (INCA) [11].

2.4.3. Productivity and Yield Variables

Regarding the productivity and yield variables, 40 fruits were chosen at random for each treatment throughout the productive cycle. The average equatorial diameter (cm) and fresh mass (g fruit⁻¹) of the fruits were performed using a caliper and a Sartorius digital BSA 124S Max 320 g scale, respectively. To find the yield of each treatment, the total production of each experimental plot was divided by the total area.

2.4.4. Economic Evaluation

The economic parameters that were taken into account to carry out the economic evaluation were: production cost, production value, and profit and profitability. The evaluation of the aforementioned parameters was carried out as follows: the cost of production (CP) in \$/ha was taken into account using the direct and indirect costs of production;

production value VP (\$/ha) was calculated using the yield for the sale price according to the national price list of protected crops for state facilities; Profit (G), in \$/ha, was the value of production less the cost of production, and profitability(R) was determined by means of the profit divided by cost of production. [13].

2.5. Statistical Analysis

Statistical analysis was developed from the evaluated parameters of plant growth and development, being height (m) and thickness (mm), and dry mass per plant organ, as well as foliar and productive NPK, being equatorial diameter (cm), weight (g) of the fruits, and yield (t/ha^{-1}). The experimental data for each variable studied were subjected to a simple classification analysis of variance (ANOVA), when there were significant differences. Comparisons of means were made according to Duncan's multiple range test for $p \leq 0.05$. The results were evaluated using the statistical package Stagraphics Centurion. XV.v15.2.14 and were graphed with the Microsoft Excel 2010 program, Santiago de Cuba. Cuba.

3. Results

3.1. Plant Growth and Development

Table 2 shows the results of the height and average thickness of the tomato plants; these variables were analyzed at 25, 50, and 75 days after transplantation and pre-inoculation with the *Rhizobium* strains under protected conditions. The evaluated variables showed a greater increase in the average height and thickness of the plants inoculated with *R. etli* CE-3 and *R.l*-SCR compared to the other strain and the control treatment, although at 25 and 75 (ddt), there were no significant differences between the treatments or in the thickness of the stem during the first evaluation.

Table 2. Height (m) and thickness (mm) of the plants inoculated with *Rhizobium*.

Treatments	First Measurement (25 d.a.t.)		Second Measurement (50 d.a.t.)		Third Measurement (75 d.a.t.)	
	Height (m)	Thickness (mm)	Height (m)	Thickness (mm)	Height (m)	Thickness (mm)
(Control) not inoculated	0.27 ^c	10.8 ^c	0.69 ^d	14.3 ^d	1.02 ^c	18.9 ^d
<i>R. etli</i> CE-3	0.43 ^a	12.1 ^a	0.84 ^a	16.5 ^a	1.21 ^a	21.3 ^a
<i>R.l</i> SCR	0.36 ^b	11.7 ^b	0.77 ^b	15.8 ^b	1.13 ^b	19.7 ^b
<i>R.l</i> Semia-4048	0.31 ^{b,c}	11.2 ^{b,c}	0.73 ^c	15.1 ^c	1.07 ^c	19.1 ^c
ESM	0.0105	0.0807	0.0451	0.116	0.033	0.126

T1 (Control) not inoculated; T2 (*R. etli* CE-3), T3 (*R.l* SCR), and T4 (*R.l* Semia-4048). Means with different letters have significant differences ($p \leq 0.05$).

3.2. Dry Mass by Plant Organ and Foliar NPK Content

The results shown in Table 3 referring to the parameters of dry mass per organ of the tomato plants inoculated with *Rhizobium* at 80 days after transplantation show significant differences between the treatments and the organs of the evaluated plants. The highest values of dry mass were evidenced in the leaves, with the treatment inoculated with the *R. etli* CE-3 strain being the one that showed the best result. For the foliar contents of NPK present in the plants inoculated with *Rhizobium*, T2 (*R. etli* CE-3) obtained the best results and the statistical analyses showed significant differences between the treatments for N while the values for P and K did not show significant differences for the treatments T3 (*R.l* SCR) or T4 (*R.l* Semia-4048), although the values for these two treatments were superior with respect to the production control.

Table 3. Dry mass (g Plant⁻¹) and NPK Content (g kg⁻¹) of the foliar contents of plants inoculated with *Rhizobium*.

Treatments	Dry Mass (g Plant ⁻¹)			NPK(g kg ⁻¹) Foliar		
	Leaf	Stem	Root	N	P (P ₂ O ₅)	K (K ₂ O)
(Control) not inoculated	10.35 ^d	3.84 ^d	1.62 ^d	2.585 ^d	0.105 ^c	0.595 ^c
<i>R. etli</i> CE-3	18.69 ^a	6.65 ^a	3.41 ^a	3.597 ^a	0.187 ^a	0.823 ^a
<i>R.l</i> SCR	16.08 ^b	5.02 ^b	2.18 ^b	3.285 ^b	0.125 ^b	0.685 ^b
<i>R.l</i> Semia-4048	11.00 ^c	4.40 ^c	2.05 ^c	3.012 ^c	0.108 ^b	0.678 ^b
ESM	0.1067	0.3431	0.1167	0.1124	0.0436	0.0253

T1 (Control) not inoculated; T2 (*R. etli* CE-3), T3 (*R.l* SCR), and T4 (*R.l* Semia-4048). Means with different letters have significant differences ($p \leq 0.05$).

The results shown in Table 4, When analyzing the evaluated parameters of the crop yield, equatorial diameter and fruit weight, it was observed that the highest values were recorded in treatments 2 and 3, which were the plants inoculated with *R. etli* CE-3 and *R.l* SCR, respectively. In the same way, these treatments had yields reaching values of 81.16 and 77.25 t/ha⁻¹, which were greater than those observed for the other treatments.

Table 4. Equatorial diameter (cm), weight (g) of the fruits, and yield in (t/ha⁻¹) for plants inoculated with *Rhizobium*.

Treatments	Equatorial Diameter (cm)	Fruit Weight (g)	Yield (t/ha ⁻¹)
(Control) not inoculated	5.1 ^d	141.33 ^d	70.20 ^d
<i>R. etli</i> CE-3	7.9 ^a	235.25 ^a	81.16 ^a
<i>R.l</i> SCR	6.5 ^b	155.43 ^b	77.55 ^b
<i>R.l</i> Semia-4048	5.8 ^c	150.36 ^c	72.25 ^c
ESM	0.1426	0.3261	0.1943

T1 (Control) not inoculated; T2 (*R. etli* CE-3), T3 (*R.l* SCR), and T4 (*R.l* Semia-4048). Means with different letters have significant differences ($p \leq 0.05$).

3.3. Economic Evaluation

Table 5 shows the economic results evaluated using production cost, production value, and Profit and Profitability for the application of *Rhizobium* in tomato cultivation. The treatments evaluated presented significant differences, but treatment two, inoculated with *R. etli* CE-3, was the one that performed best with respect to the other two strains evaluated and the uninoculated control, having a yield of 1.48. In this experiment, no monetary losses were quantified despite the fact that the evaluated treatments did not behave in the same way.

Table 5. Economic evaluation in thousands of Pesos of Cuban currency.

Treatments	Economic Indicators in Cuban Currency Thousands.			
	C.P	VP	P	P
(Control) not inoculated	10.33	16.8	6.47	0.62
<i>R. etli</i> CE-3	10.41	25.92	15.51	1.48
<i>R.l</i> SCR	10.39	24.88	14.49	1.39
<i>R.l</i> Semia-4048	10.39	23.12	12.73	1.22

T1 (Control) not inoculated; T2 (*R. etli* CE-3), T3 (*R.l* SCR), and T4 (*R.l* Semia-4048). Means with different letters have significant differences ($p \leq 0.05$). C.P (Cost of production), VP (Value of production), P (Profit), and P (Profitability).

4. Discussion

In this research, the results show the efficiency of *Rhizobium* and the strains used. The treatments inoculated with the *R. etli* CE-3 and *R.l* SCR strains were the ones that

achieved the best results with respect to the evaluated variables with respect to that of the *R. l* Semia-4048 strain and the uninoculated control. The results obtained show the positive effect of applying *Rhizobium* strains to tomato plants.

In previous research (regardless of the methodology), it has been described that the inoculation of *Rhizobium* has managed to improve the growth and development of tomato (*Solanum lycopersicum* L.) seedlings and there is reference to the fact that *Rhizobium* is a microorganism capable of fixing nitrogen asimbiotically and dissolve phosphates to favor the nutrition of the tomato seedling, a quality that makes it a microorganism with PGPR capacity [6]. A previous study has also shown that, in the cultivation of lettuce (*Lactuca sativa* L.), positive results were obtained for the dry weight of leaves, stems, and shoots, as well as in the length of the root and height of the plant with the application of the strain *R. etli* [8].

The results obtained in this investigation for each evaluated variable could be given by the capacity of these Rhizobacteria, which, when interacting with the roots of non-legume seedlings, are attracted by substances emitted by the root, allowing for the movement of the bacteria towards the root of the plant seedling and initiating a beneficial symbiosis, a process that occurs through chemotaxis mechanisms related to the presence of flagella, chemoreceptors, and genetically encoded regulatory systems [14]. Other benefits that are conferred to *Rhizobium* are the direct action it exerts on the production of phytohormones, a process that occurs naturally. These phytohormones include five known groups of compounds, being auxins, ethylene, gibberellins, cytokines, and abscisic acid, each of which has a direct action on plant growth and development [9].

When analyzing the data of the evaluated parameters in the tomato plants, the efficiency of this rhizobacteria in the inoculated treatments with respect to the control is evidenced, These results were corroborated by statistical analyses that showed a significant difference ($p < 0.05$) that affirms that this PGPR has the capacity to produce substances that promote plant development in non-legume plants [15]. These growth-regulating substances stimulate the density and length of the root hairs and lateral roots, thus developing the capacity to absorb water and nutrients, as evidenced in the greater measures of growth parameters, achieving a beneficial effect on the dry weight of the aerial part the crop yield [16].

In this research, when analyzing the economic parameters of production cost, production value, and profit and profitability from the *Rhizobium* inoculation with respect to the control, it was shown that the use of biofertilizers in tomato plants not only had a positive response in the other parameters evaluated, but also in the economic ones, as none of the evaluated treatments created economic losses and instead the treatments inoculated with the *Rhizobium* strains were the ones that obtained the best results. Based on the results of this research, it is evident that with the application of *Rhizobium* to tomato plants, favorable results in production are achieved, which contributes to reducing the use of nitrogenous fertilizers, thereby remedying the problems of soil and water contamination and decreasing production costs.

Today, the main challenge of modern agriculture is the production of high-quality, ecologically safe, and economically affordable food. The use of biofertilizers makes it possible to work on a sustainable agriculture approach based on the use of beneficial microorganisms, which can ultimately guarantee the high production of agricultural crops with lower costs, a higher biological quality of the crops, and an increase in the biological activity of the soil based on the care of the environment.

5. Conclusions

The results obtained showed a positive effect on the indicators evaluated in the plants inoculated with the *Rhizobium* strains with respect to the uninoculated control. The best results were obtained in the tomato yield (*Solanum lycopersicum* L.) for plants with the inoculation of the *R. etli* CE-3 strain.

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