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Research Article

Impact of *Bacillus subtilis* as a Growth Promoter in the Cultivation of Tabasco Chili Pepper (*Capsicum frutescens*)

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The growth and production of Tabasco chili are limited by various fertilization, edaphoclimatic and biotic factors. This study evaluated the influence of *Bacillus subtilis* on the growth of *Capsicum frutescens* cultivars by characterizing plant conditions before and after treatment. The research was carried out in the Mayo river basin, in the district and province of Moyobamba, San Martín, Peru, between 76.98° and 76.99° west longitude and 6.05° and 6.06° south latitude, according to the WGS 1984 geographical coordinate system. The experiment followed a completely randomized block design with four treatments and three replicates each. A total of 48 Tabasco chili plants were planted at 40 cm spacing per experimental unit. Field data on plant height and leaf length were processed and presented using box plots and numerical tables. The results showed that after 30 days, plants treated with *B. subtilis* reached heights of 18.0 cm and leaf lengths of 3.9 cm. After 60 and 90 days, plants treated with *B. subtilis* reached heights of 43.2 and 110 cm, respectively, with corresponding leaf lengths of 16.2 and 23 cm. It was concluded that the application of *B. subtilis* at a concentration of 250 mL *B. subtilis* + 750 mL water significantly enhanced the growth of Tabasco chili as measured by plant height and leaf length at different stages of development.

Keywords: *Bacillus subtilis*; *Capsicum frutescens*; leaf length; plant height; randomized block; Tabasco chili

1. Introduction

As modern agriculture advances, the use of agrochemicals has become essential for crop production, with 2 million tons of these products applied globally to boost yields and minimize losses due to diseases and pests [1]. Organophosphorus pesticides are widely used for their effectiveness in reducing infections and increasing yield, though their long-term overuse poses risks to the environment, soil, water, and animal health [2]. Among PGPMs, the *Bacillus* genus stands out for its ability to degrade a wide range of organic materials and adapt to extreme conditions, performing processes like nitrogen fixation, nitrification, and denitrification. *Bacillus* species promote plant growth directly through these processes and indirectly by producing substances that act as pathogen antagonists or induce resistance in plants. In the context of Tabasco chili (*Capsicum frutescens*), the plant's growth is hindered by phytosanitary issues such as anthracnose, caused by *Colletotrichum scovillei*, with resistant cultivars being the most effective solution [3]. Research on good agricultural practices (GAPs) for *C. frutescens* found promising results, with an average score of 2.70 across 10 agronomic parameters in 100 farmers,

emphasizing the potential for regional production growth using a strengths-opportunities strategy [4]. Furthermore, agroclimatic conditions are crucial, as evapotranspiration measurements in Brazil's Northeast show maximum values of 5.6 mm with variable crop coefficients during flowering and fruit development [5].

Tabasco chili production can be optimized by adjusting planting density and nitrogen fertilization, with studies showing that mechanized and manual harvests yield similar results [6, 7]. In controlled environments, using substrates like 40% hillside soil and 40% chicken bedding under drip irrigation significantly improves productivity [8]. In Indonesia, yields ranged from 3084 to 6423 kg/ha, demonstrating the potential for high profitability [7], and greenhouse cultivation uses 5% less water than open-field cultivation, showing more efficient water use [9]. However, challenges like reduced agricultural diversity and greater food dependency require sustainable models for further expansion [10].

The main objective of the research was to evaluate the influence of *Bacillus subtilis* on the growth of *C. frutescens* cultivars by characterizing the condition of the plants before and after treatment. Subsequently, differences in plant heights and leaf lengths were determined and evaluated. The research was developed in response to the need to generate ecological alternatives to replace agrochemicals in the cultivation of *C. frutescens* to improve its growth and productivity. It is known that *C. frutescens*, commonly known as "aji tabasco" in Peru, is highly profitable when exported to North America.

The development of biostimulants based on *B. subtilis* and *Bacillus velezensis* offers sustainable alternatives to pesticides. These strains improve plant growth, mitigate abiotic stress, and act as biocontrol agents, reducing the need for chemicals in farming [11, 12]. Strains like *B. subtilis* EA-CB0575 and ER-08 have proven effective in promoting growth and enhancing resistance to diseases in an environmentally friendly manner [13–15]. *Bacillus subtilis* also benefits from its interaction with the plant's rhizosphere, promoting growth through nutritional improvements, hormonal modulation, and antimicrobial production, which is critical for sustainable agriculture [16–18]. Research also highlights the effectiveness of *B. subtilis* in controlling pathogens like *Fusarium oxysporum*, with strain FJ3 showing 92% inhibition and a 24.01% increase in chickpea growth Jan et al. [19], and its potential use as a biocontrol agent for pests like *Tenebrio molitor* [20].

Given its beneficial effects on plant development, *B. subtilis* and similar microorganisms have been widely studied for their role in promoting chili pepper seedling growth. These microorganisms enhance plant height and leaf production by producing indoleacetic acid (IAA), a plant hormone that stimulates growth, and by solubilizing phosphates, which increases nutrient availability in the soil [21]. As a result, plants can absorb essential nutrients more efficiently, leading to healthier growth and development [22–24].

Agroecology, which promotes sustainable farming practices such as organic farming and reducing chemical inputs, fosters biodiversity, resilience, and food sovereignty [25–27]. Sustainable practices such as drip irrigation and biofertilizers are increasingly essential, especially with a projected global population of 9.1 billion by 2050 [28]. Alternatives like crop rotation and manure can further enhance sustainability [29], with principles like recycling, biodiversity, equity, and responsible governance being central to resilient systems [30]. Sustainable intensification aims to improve environmental outcomes, while boosting agricultural production [31], with these approaches fostering a socio-ecological transition [32].

Numerous studies have demonstrated the potential of *B. subtilis* as a plant growth-promoting rhizobacterium and biological control agent, which is particularly relevant in the context of Amazonian agriculture, where there is a strong emphasis on enhancing productivity through sustainable and environmentally low-impact practices. Chowdhury et al. [33] report that *Bacillus amyloliquefaciens* FZB42, a close relative of *B. subtilis*, promotes plant development through the production of secondary metabolites, nutrient competition in the rhizosphere, and the induction of systemic resistance.

Similarly, Hashem et al. [34] highlight that *B. subtilis* not only enhances nutrient uptake and plant growth, but also strengthens plant responses against biotic stress, including diseases and pathogens. Taken together, these findings support the use of *B. subtilis* as an effective and sustainable biotechnological alternative to improve the yield of Amazonian crops such as Tabasco pepper (*C. frutescens*), in alignment with ecosystem conservation.

Although research on the use of *B. subtilis* in agriculture is expanding, few studies have specifically focused on its effects during the early growth stages of *C. frutescens* under local agroecological conditions. Meanwhile, to date, few field investigations have examined the growth-promoting effects of *B. subtilis* on *C. frutescens* under the conditions of the Peruvian Amazon. This study aims to fill that gap by presenting experimental data on the growth-promoting effects of *B. subtilis* on the Tabasco pepper crop, thereby, contributing to the development of sustainable alternatives to chemical inputs and enhancing current knowledge on the use of biostimulants in high-value tropical crops.

2. Materials and Methods

2.1. Study of the Site. The study area is located in the Mayo river basin, in the district and province of Moyobamba in the department of San Martin in Peru, between parallels 76.98° and 76.99° west longitude and between 6.05° and 6.06° south latitude, according to the WGS 1984 geographical coordinate system. The research was carried out specifically in the experimental field of the "La Tipishca" Production Center of the Alto Mayo Public Technological Higher Education Institute, located at coordinates −6.05382° S and −76.98603° W. The experimental field had a total area of 36.0 m^2. The treatments during the experimental stage lasted for 90 days. The materials and equipment used in the research included a balance, 1000 and 500 mL test tubes, *B. subtilis*, rock phosphate, lime, organic matter, cattle manure, peat, humus, aji Tabasco, and personal protective equipment.

A strain of *B. subtilis* ATCC 6051 was used, selected for its proven ability to promote plant growth in Solanaceous species. The bacterial suspension was standardized to 10^6 CFU/mL

Table 1: Analysis of the compounds present by *Bacillus subtillis*.

Magazine	Quartile	Descripción	Authors
AMB express	Q1	The application of biotransformation techniques with *B. subtilis* can enhance the biological activity of flavonoids through the generation of bioactive metabolites	Gendy et al. [38]
Frontiers in microbiology	Q1	*B. subtilis* strains have a spectrum of action against foodborne or phytopathogenic flora, as well as a history of safe use in food	Caulier et al.[39]
Food bioengineering	Q2	*B. subtilis* presents use as "microbial cell factories" due to the bioactive compounds it produces and have the potential to effectively solve future health, environmental, and energy problems	Miyazawa et al.[40]
Frontiers in microbiology	Q1	Volatile organic compounds derived from *B. subtilis* strain SYST2 promote tomato plant growth by activating growth hormone activity	Tahir et al. [41]
Microbiological research	Q1	*B. subtilis* PMB102 suppresses Alternaria leaf spot by producing antifungal metabolites, inhibiting vegetative growth of *A. brassicicola* ABA-31	Wu et al. [42]

using nutrient agar plate counts. Plant height was measured using a Stanley PowerLock metal measuring tape with an accuracy of ±1 mm. The tape was positioned at the base of the stem at soil level and extended to the apical point of the plant, excluding flowers or fruits. To ensure accuracy, three measurements were taken per plant in different directions, and the average value was calculated.

Leaf length was measured using a Mitutoyo digital caliper (Model 500-196-30, accuracy of 0.01 mm). For each plant, three fully developed leaves were selected, and measurements were taken from the base of the petiole to the leaf apex, with the average used for analysis.

The experimental soil exhibited typical characteristics of the Moyobamba province, with a loam-clay texture (60% sand, 30% clay, and 10% silt), pH ranging from 6.2 ± 0.3 to 6.5–7.0, and organic matter content between 3.2%–4%. Optimal levels of nitrogen (20–30 mg/kg), phosphorus (15–25 mg/kg), and potassium (150–200 mg/kg) were also recorded.

Climatic conditions during the study included average temperatures between 24 and 28°C (mean 25 ± 2°C), relative humidity ranging from 70%–80%, and annual precipitation between 1200 and 1500 mm (with a total recorded value of 1400 mm). These parameters align with the agroecological requirements for cultivating *C. frutescens* in the Peruvian Amazon.

2.2. Research Design. The experiment was conducted under a completely randomized block design (CRBD), where four treatments were evaluated with three replications, and each replication consisted of a set of individual plants. The experimental units were the Tabasco chili pepper plants (*C. frutescens*), each of which received a specific *B. subtilis* treatment at a predetermined dosage. A total of 48 individual plants were used, planted 40 cm apart within each experimental unit. Data on plant height and leaf length were collected in the field and presented through box plots and numerical tables. Treatments consisted of T1: 150 mL of *B. subtilis* + 850 mL of H_2O, T2: 200 mL of *B. subtilis* + 800 mL of H_2O, T3: 250 mL of *B. subtilis* + 750 mL of H_2O, and T4: 1 L of H_2O (control).

Plant height and leaf length were measured before and after inoculant application at 30, 60, and 90 days using the spray method, and recorded directly in the field. A total volume of 100 mL per plant was used for each spray application, uniformly distributed over the aerial parts. Applications were carried out during afternoon hours. Soil preparation included plowing to define the experimental plots and the construction of four 4.00 × 1.50 m planting beds. A basal dressing was applied using a mixture of organic and inorganic fertilizers (rock phosphate, lime, organic matter, and cattle manure), followed by a final plowing to incorporate the fertilizers into the soil prior to planting.

Due to its beneficial effects on plant growth and stress reduction, *B. subtilis* was used as the inoculant in this study. The strain used was previously cultured under laboratory conditions and originally isolated from agricultural soil in the Alto Mayo region, San Martín, Peru. The culture was identified and activated in specific growth media prior to being diluted for application. Although a specific cell count per milliliter of the stock solution was not performed, the same preparation batch was used for all treatments to ensure consistency in relative concentration. This microorganism enhances plant development and mitigates biotic stress by inducing systemic resistance, antibiosis, and competitive exclusion, acting as both a biofertilizer and a biopesticide [35]. It also increases plant tolerance to drought and salinity stress, improving productivity without the harmful effects of chemical fertilizers [36]. *Bacillus subtilis* promotes plant growth and protects crops by improving nutrient availability and suppressing pathogens; however, its success depends on effective root colonization and the specific characteristics of the bacterial strain [37]. It also presents compounds of scientific interest (Table 1).

2.3. Seed Germination Process. Ten kilograms of Tabasco chili were collected, obtaining 1 kg of seeds manually. After washing

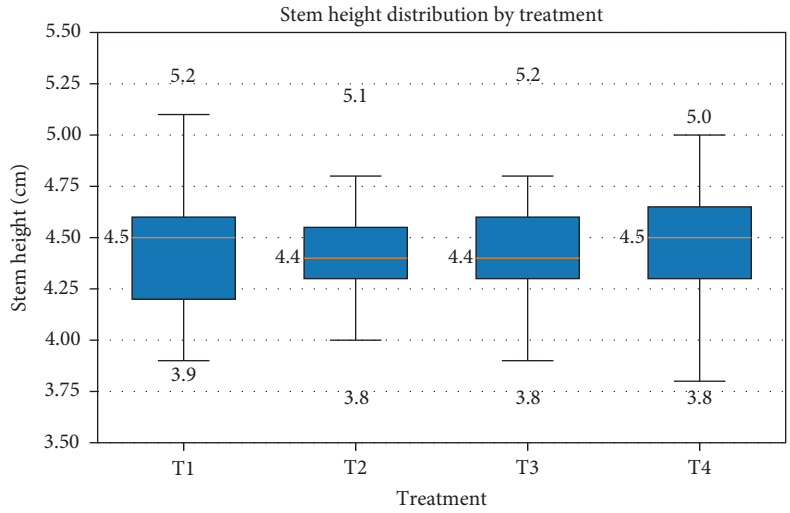

FIGURE 1: Initial height status of *Capsicum frutescens* plants before *Bacillus subtilis* application according to treatment subplots.

and drying the seeds for 24 h, a germination test was carried out, which showed 99% viability. A substrate was prepared with 600 g of peat and 400 g of humus, mixed with 1 L of rainwater, and the seeds were sown in germination trays with a total weight of 2.2–3 kg. Fourteen days after sowing, initial measurements of growth variables, such as plant height and leaf length, were taken using appropriate instruments and recording the data in established formats [23, 43].

2.4. Preparation of Bacillus subtilis Solution. A homogeneous 1000 mL solution was prepared using biological input and rainwater in different proportions. For this purpose, various glass and plastic materials, such as test tubes and tanks, were used to measure and mix specific amounts of the biological input and rainwater. The final solution was stored in spray containers for later application. The distribution of each treatment as follows:

$$\begin{aligned}
&T1: 150\,\text{mL BS} + 850\,\text{mL } H_2O \rightarrow 1000\,\text{mL};\\
&T2: 200\,\text{mL BS} + 800\,\text{mL } H_2O \rightarrow 1000\,\text{mL};\\
&T3: 250\,\text{mL BS} + 750\,\text{mL } H_2O \rightarrow 1000\,\text{mL};\\
&T4: 1\,\text{L rainwater (control)}.
\end{aligned}$$

The biological treatment was applied in multiple periods, during the afternoon hours, by spraying with a hand sprayer. After each application, plant growth variables, such as plant height and leaf length, were measured. The levels of these variables were compared before and after the application of the biological treatment, using statistical analysis to evaluate their effect on crop growth.

2.5. Statistical Analysis. In the analysis of the data, descriptive and inferential statistical techniques were used with the aid of statistical software for the visual representation of the data by means of graphs and tables. Compliance with the necessary statistical assumptions was previously verified through corresponding tests. Subsequently, an analysis of variance (ANOVA) was performed at a 5% significance level to determine if there were significant differences between the treatments evaluated, and Duncan's multiple comparisons test was applied to identify the treatment that presented the best results.

3. Results and Discussions

3.1. Initial State of Plant Height. The greatest dispersion was recorded in subplot T1, where 50% of the plants had heights between 4.2 and 4.8 cm. In contrast, subplots T2 and T3 showed a higher concentration and lower dispersion, with plant heights ranging from 4.15 to 4.65 cm in T2 and 4.7–4.25 cm in T3. In addition, low heights of up to 3.8 cm were observed in subplots T2, T3, and T4, and high heights of up to 5.2 cm were observed in T1 and T3, these being the lowest and highest heights in the data set. The median heights were equal in subplots T1, T2, and T4 (4.45 cm), and 4.4 cm in T3 (Figure 1).

3.2. Initial State of Plant Leaf Lengths. The greatest dispersion was recorded in subplot T3, where 50% of the plants had leaf lengths between 1.75 and 2.1 cm. In contrast, subplot T1 showed higher concentration and lower dispersion, with leaf lengths between 1.8 and 2.0 cm. In addition, low leaf lengths of up to 1.5 cm were observed in subplots T1, T2, and T4, and high leaf lengths of up to 2.3 cm in T4, representing the lowest and highest values in the data set. Median leaf lengths were equal in subplots T2 and T3 (2.0 cm), while they were 1.9 cm in subplots T1 and T4 (Figure 2).

3.3. Status of Capsicum frutescens Plants After Bacillus subtilis Application

3.3.1. Plant Height Status 30, 60, and 90 Days After the Application of Bacillus subtilis. The highest plant heights were observed, with the application of 250 mL of *B. subtilis* + 750 mL of H_2O, corresponding to treatment T3, where a greater dispersion of 50% of the plant population was observed with heights between 16.25 and 17.7 cm and an interquartile range of 1.45 cm. The lowest plant heights were recorded in the control subplot T4 (1 L H_2O), while subplot T1 showed a

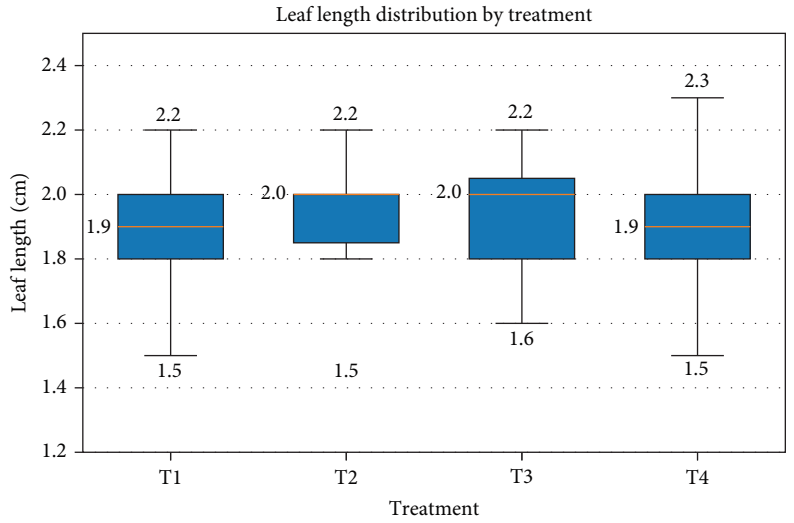

Figure 2: Initial leaf length status of *Capsicum frutescens* plants before *Bacillus subtilis* application according to treatment subplots.

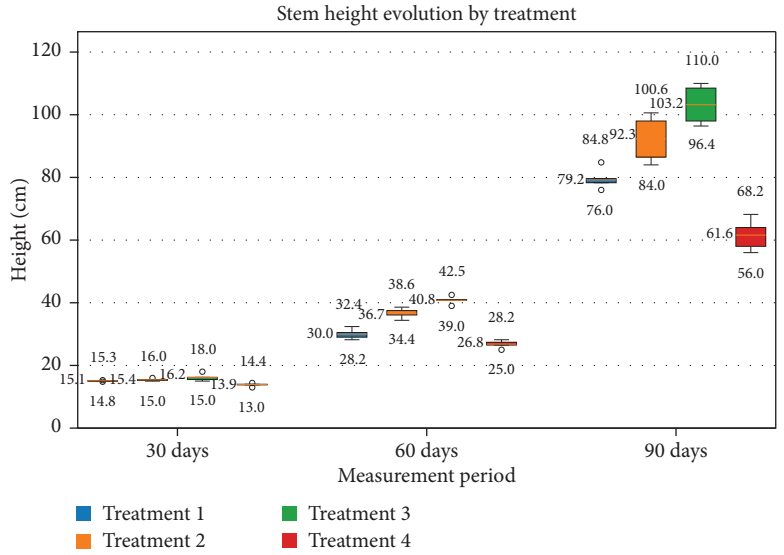

Figure 3: Height status of *Capsicum frutescens* plants 30, 60, and 90 days after *Bacillus subtilis* application.

higher height concentration, with 50% of the population in an interquartile range of 0.55 cm. The highest height of 18.0 cm was recorded in T3 and the lowest height of 13.0 cm in T4 (Figure 3). The use of plant growth-promoting bacteria is known to be a way of partially replacing the use of chemical fertilizers on commercial crops, which not only make production more expensive but also have a negative impact on the environment [44].

The highest plant heights were observed, as at 30 days, with the application of 250 mL of *B. subtilis* + 750 mL of H_2O, corresponding to treatment T3. In this treatment, a greater dispersion of 50% of the plant population was observed with heights between 30.6 and 41.0 cm and an interquartile range of 10.4 cm. The lowest heights were recorded in the control subplot T4 (1 L H_2O), where a greater concentration of plant height was evident, with 50% of the population in an interquartile range of 2.6 cm. The highest height of 43.2 cm was recorded in T3 and the lowest height of 24.3 cm in T1 (Figure 3). Lara-Capistrán et al. [45] showed that *C. frutescens* plants inoculated with *B. subtilis* bacteria showed a significant increase in height, stem diameter, number of leaves, buds and flowers and leaf area, even more than plants that had been chemically fertilized. The increase in morphological parameters of *C. frutescens* with *Bacillus subtilis* may be due to the fact that *B. subtilis* has the ability to produce low molecular weight organic acids that allow the release of soluble phosphorus from the soil; this macroelement is used by plants for photosynthesis, degradation of energy and carbon sources, allowing them to achieve greater plant growth [46]. *Bacillus subtilis* also promotes IAA, a plant hormone capable of inducing cell elongation. Meanwhile, these bacteria have a high capacity for adhesion and colonization of plant roots, which has a greater effect on plant [47].

The greatest plant heights were again observer with the application of 250 mL of *B. subtilis* + 750 mL of H_2O,

Table 2: Duncan test of treatments for leaf length of *Capsicum frutescens* plants at 30, 60, and 90 days after *Bacillus subtilis* application.

Treatments	*N*	30 days			60 days				90 days			
		F										
		4539.3555			818.6935				1764.1769			
		p-Value										
		2.91E−07			8.88E−06				1.92E−06			
		Subset			Subset				Subset			
		1	2	3	1	2	3	4	1	2	3	4
T4	12	a			a				a			
T1	12		ab			ab				ab		
T2	12		ab				bc				bc	
T3	12			b				c				c

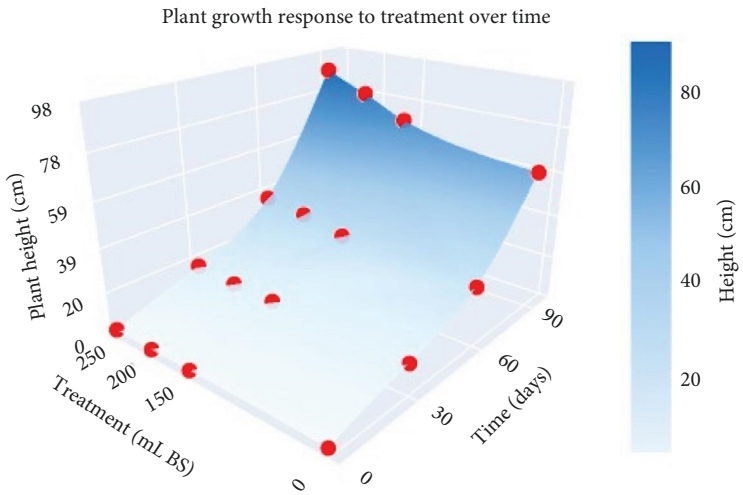

Figure 4: Average height distribution of *Capsicum frutescens* plants pre and post *Bacillus subtilis* application.

corresponding to treatment T3, both at 30 and 60 days. In this treatment, a greater dispersion of 50% of the plant population was observed, with heights between 79.0 and 99.0 cm and an interquartile range of 20.0 cm. The lowest heights were identified in the control subplot T4 (1 L H_2O), where there was also a greater concentration of heights, with 50% of the population in an interquartile range of 13.7 cm. The highest height of 110.0 cm was recorded in T3 and the lowest height of 50.4 cm in T4 (Figure 3). Mejía-Bautista et al. [48] reported that the *B. subtilis* strain led to significant increases in the height of *Capsicum chinense* at both 60 and 140 days, with mean values of 21.4 and 129.7 cm, respectively. In comparison, the present study recorded an average plant height of 110 cm at 90 days, using a mixture of 250 mL of *Bacillus subtilis* and 750 mL of H_2O. Similarly, Cárdenas-Flores et al. [49] reported heights exceeding 40 cm in *Capsicum annuum* for the Valkiria and Sven varieties evaluated at 60 days. These findings align with the results of the current study, in which an average height of 43.2 cm was observed at the 60-day mark, confirming the consistency of our data with previously published studies.

According to the plant height averages for the different treatments before and after *B. subtilis* application (Figure 4), it was verified that, at 0 days, the height averages were relatively similar. However, significant differences were recorded in the means after inoculant application at 30, 60, and 90 days, with the T3 curve always above the other treatments. The application of 250 mL of *B. subtilis* + 750 mL of H_2O resulted in the highest mean height of *C. frutescens* plants, while the T4 (control) treatment showed significantly lower height means compared to the other treatments.

3.4. Influence of Bacillus subtilis on the Length of Plant Leaves. The Duncan test (Table 2) shows that treatment T4 had the lowest mean leaf length (group "a"), suggesting that this treatment was the least effective in promoting growth at this early stage. Treatments T1 and T2 were grouped in the same subset (group "ab"), indicating that there were no significant differences between them, although both had a greater leaf length than T4. Treatment T3 had the longest leaf length and was placed in a different subset (group "b"), indicating that it was significantly more effective than the other treatments for this variable at 30 days.

At this stage, treatment T4 continued to have the shortest leaf length (group "a"), indicating limited growth compared to the other treatments. Treatment T1, classified in group 'ab', showed an intermediate length, indicating moderate growth.

Table 3: Duncan test of treatments for height of *Capsicum frutescens* plants at 30, 60, and 90 days after *Bacillus subtilis* application.

		30 days			60 days				90 days			
					F							
		369.1294656			110.731456				2.489697784			
Treatments	*N*				*p*-Value							
		4.33E-05			4.61E-04				1.90E-01			
		Subset			Subset				Subset			
		1	2	3	1	2	3	4	1	2	3	4
T4	12	a			a				a			
T1	12		ab			ab				ab		
T2	12		ab				bc				bc	
T3	12			b				c				c

Treatment T2 was classified in subgroup "bc" and showed an increase in leaf length compared to T4 and T1, although still lower than T3. Finally, T3 was the most effective treatment at this stage, grouped in subset "c," indicating significant differences with respect to all other treatments.

The results show a similar trend to that observed at 60 days. T4 again showed the lowest leaf length (group "a"), confirming its lower efficiency at this stage of the study. Treatment T1, classified in group "ab," showed a greater leaf length than T4, but its yield was lower than that of T2 and T3. Treatment T2 was grouped in the "bc" subset and showed a significant increase in leaf length, although still lower than T3. Finally, T3 was the most effective treatment, classified in group "c", showing significant differences with the others and achieving the highest leaf length observed at 90 days.

The study reveals that the application of *B, subtilis* at a concentration of 250 mL BS + 750 mL water has a significant positive impact on the growth of aji tabasco, as measured by plant height and leaf length at various stages of development. At 30 days, *B, subtilis*-treated plants reached heights of 18.0 cm and leaf lengths of 3.9 cm, suggesting a stimulatory effect on early growth. These results agree with the findings of Leal-Almanza et al. [50], who observed similar effects in crops such as bell pepper and potato, attributed to metabolites and stimulant substances produced by *B. subtilis*.

Plants inoculated with *B. subtilis* at 60 and 90 days had heights of 43.2 and 110 cm, respectively, and leaf lengths of 16.2 and 23 cm. These increases in vegetative development support the results of Anguiano et al. [51], who highlight the importance of selecting plant growth promoting rhizobacteria (PGPR) strains with optimal metabolic profiles to maximize plant growth promotion.

The results of the study clearly demonstrate the potential of *B. subtilis* as a bioinoculant to improve the yield of Tabasco chili, coinciding with previous research on similar crops that highlights the advantages of combining PGPR with organic substrates [51]. This sustainable strategy based on beneficial microorganisms could reduce the dependance on chemical fertilizers in the production of Tabasco chili. The study provides strong evidence on the ability of *B. subtilis* to significantly promote vegetative growth of ají Tabasco, especially in terms of height and leaf development, reinforcing its potential as a biofertilizer and biological control agent in this economically important crop.

3.5. Influence of Bacillus subtilis on Plant Height. The Duncan test (Table 3) shows that after 30 days, treatment T4 showed the lowest average growth and was classified as group "a," indicating that it was significantly different from the other treatments. Treatment T3 showed the highest growth and was placed in group "b," reflecting a significantly better behavior than T4. Treatments T1 and T2 were placed in group "ab," indicating that there are no statistically significant differences between them or with the extreme treatments (T4 and T3), but they show an intermediate performance. After 60 days, the differences between the treatments became more apparent. Treatment T4 remained in group "a," maintaining the lowest average growth. T1 was placed in the "ab" group, maintaining intermediate growth. Treatment T2 was placed in group "bc," indicating that it was beginning to differentiate more from T1 and T4. T3 showed the highest average growth and was clearly positioned in group "c," being significantly different from the other treatments.

At 90 days, the differences between treatments were even more pronounced. T4 remained in group "a," again reflecting the lowest average growth. T1 showed intermediate growth and was placed in group "ab," indicating a moderate improvement. T2 was placed in group "bc," reflecting a higher performance than T1 but lower than T3. Finally, T3, with the highest average growth, was placed in group "c," indicating a significant difference compared to all other treatments.

3.6. Plant Leaf Length Status 30, 60, and 90 Days After the Application of Bacillus subtilis. The greatest plant leaf lengths were recorded with the application of 250 mL of ***B. subtilis*** + 750 mL of H_2O, corresponding to treatment T3. In this treatment, a greater dispersion of 50% of the population of plants with leaf lengths between 4.05 and 4.35 cm and an interquartile range of 0.3 cm was observed. The lowest leaf lengths were recorded in the control subplot T4 (1 L H_2O), while a higher concentration of leaf length was observed in T2 and T4, where 50% of the populations were in an interquartile range of 0.2 cm. The highest leaf length, 4.5 cm, was recorded with T3, and the lowest, 2.6 cm, with T4 (Figure 5).

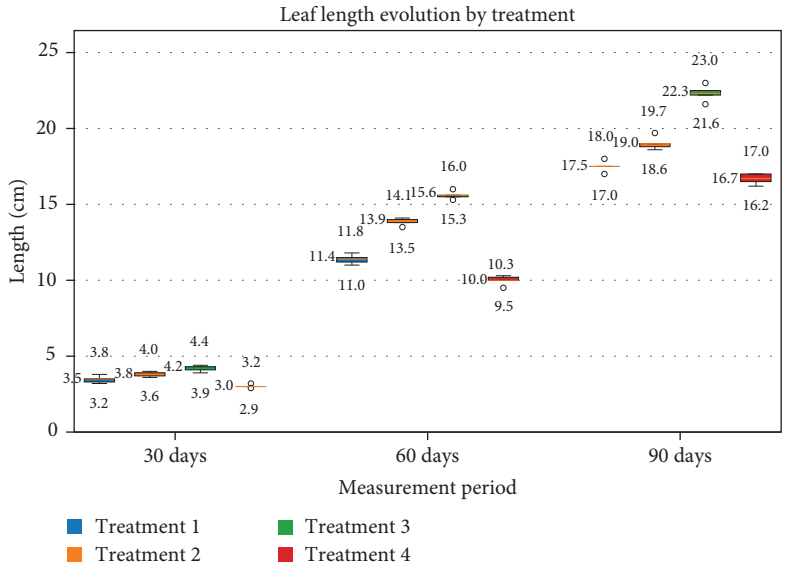

FIGURE 5: Leaf length status of *Capsicum frutescens* at 30, 60, 90 days after *Bacillus subtilis* application.

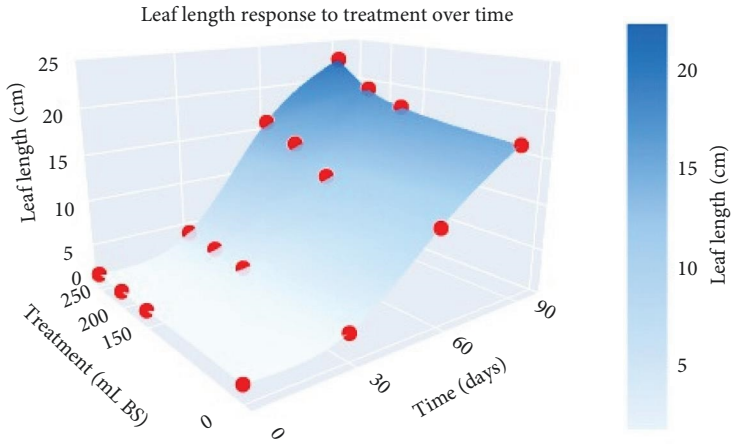

FIGURE 6: Average leaf length distribution of *Capsicum frutescens* plants pre- and post-*Bacillus subtilis* application.

The greatest leaf lengths were recorded, as at 30 days, with the application of 250 mL of ***B. subtilis*** + 750 mL of H_2O corresponding to treatment T3. At T1, a greater dispersion of 50% of the plant population was observed with leaf lengths between 11.1 and 11.9 cm and an interquartile range of 0.8 cm. The shortest leaf lengths were recorded in the control subplot T4 (1 L H_2O), while T2 showed a higher concentration of lengths, with 50% of the populations in an interquartile range of 0.35 cm. The greatest leaf length, 16.2 cm, was recorded in T3 and the least, 9.5 cm, in T4 (Figure 5).

The greatest plant leaf lengths were recorded at 30 and 60 days by the application of 250 mL ***B. subtilis*** + 750 mL H_2O, corresponding to T3, a treatment that also showed the highest concentration of 50% of the plant population with leaf lengths between 22.0 and 22.5 cm and an interquartile range of 0.55 cm. The smallest leaf lengths were recorded in the control subplot T4 (1 L H_2O), while a greater dispersion of leaf length was observed in T2, with an interquartile range of 0.95 cm. The greatest leaf length was 23.0 cm with T3, and the least was 15.8 cm with T4 (Figure 5).

According to the leaf length averages for the different treatments pre and post *B. subtilis* application (Figure 6), significant differences in leaf length means were identified at 30, 60, and 90 days, with T3 being the treatment that generated the longest leaves. The application of 250 mL *B. subtilis* + 750 mL H_2O produced the highest mean leaf length of *C. frutescens*, while T4 (control treatment) recorded significantly lower mean values compared to the other treatments.

According to the results presented, a significant increase in the height of *C. frutescens* seedlings was observed after treatment with *B. subtilis*, reaching a maximum height of 110 cm compared to only 4.5 cm in the control group. This increase in aerial biomass can be directly correlated with the accumulation of plant material; therefore, estimations of fresh and dry weight were made based on plant height (Table 4). These values were extrapolated from plant height using a previously established

Table 4: Estimated fresh and dry weight of *Capsicum frutescens* seedlings based on plant height.

Treatment	Maximum height (cm)	Estimated fresh weight (g)	Estimated dry weight (g)
Control	4.5	1.58	0.32
Bacillus subtilis	110	38.5	7.7

empirical correlation model between above-ground biomass (fresh and dry weight) and plant height. This methodology has been employed in agronomic studies where direct measurement is not feasible due to logistical constraints or to avoid the destruction of samples during growth. The responses observed in this study are consistent with those reported by Wang et al. [52], who found increases in both plant height and fresh weight of *C. annuum* following the application of *B. velezensis*. These findings reflect the biostimulant effect of microbial treatment on plant growth and biomass accumulation, likely associated with enhanced nutrient availability, phytohormone production, and improved root development induced by *B. subtilis*; It also stimulates the growth of soybean plants [53]. While Mamani et al. [54] showed averages of 72.29 and 8.88 cm in plant height and leaf number, respectively, in onion (*Allium cepa* L.) with treatments based on a formulation with *B. amyloliquefaciens*.

4. Limitations

The Impact of *B. subtilis* as a growth promoter in the cultivation of Tabasco chili pepper (*C. frutescens*) They are an alternative to improve production and performance in the Peruvian Amazon; however, only plant height and leaf length were evaluated in this study. The yield and productivity of the crop being a limitation, which is why it is suggested in the next studies to include the yield and nutritional status of the fruits of the Tabasco pepper; Likewise, establishing sowing in different periods and times of the year in such a way allows us to have more complete information. However, the height and number of leaves are morphological indicators of crop development and yield.

5. Conclusions

The information presented shows that, before applying *B. subtilis*, the height and length of the leaves of *C. frutescens* were similar 15 days after sowing, with no significant differences between treatments. The highest average height was 4.48 cm (T1) and the lowest was 4.44 cm (T3). The average maximum leaf length was 1.93 cm (T2 and T3), and the minimum was 1.89 cm (T1 and T4). In general, plants exhibited uniform heights and leaf lengths. The application of 250 mL *Bacillus subtilis* + 750 mL of H_2O promoted maximum growth of *C. frutescens* plants, reaching heights of 18.0 cm and leaf lengths of 3.9 cm at 30 days, 43.2 cm and 16.2 cm at 60 days, and 110.0 cm and 23.0 cm at 90 days. In contrast, the control treatment with 1 L H_2O resulted in the least growth, with plant heights of 13.0, 25.0, and 50.4 cm and leaf lengths of 2.6, 9.5, and 15.8 cm at 30, 60, and 90 days, respectively. With 95% confidence, it was determined that the four treatments studied influenced the height and leaf length of *C. frutescens* at 30, 60, and 90 days after *B. subtilis* application. Duncan's test showed that the control treatment (1 L of H_2O) resulted in Tabasco plants with lower height and leaf length compared to treatments containing *B. subtilis*. The most optimal treatment was T3 (250 mL *Bacillus subtilis* + 750 mL H_2O), which had the highest concentration of *B. subtilis*, promoting the greatest heights and leaf lengths. Given these findings, future research should explore the long-term agronomic impact of *B. subtilis* under diverse field conditions, as well as evaluate the efficacy of alternative *Bacillus* strains or consortia to enhance the generalisability and scalability of this bioinoculant approach. Studies incorporating soil microbiome analyses and post-harvest yield assessments would also provide a more holistic understanding of its agricultural potential.

Data Availability Statement

Data used and analyzed during the course of this research are available upon reasonable request to the corresponding author. The authors declare that there are no additional data to be shown beyond those presented in the body of the article and in the included tables and figures.

Conflicts of Interest

The authors declare no conflicts of interest.

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