




Production of chile de árbol (*Capsicum annuum* L.) seedling with designation of origin in Yahualica, Jalisco

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ABSTRACT

Objective: Different chile de árbol (*Capsicum annuum* L.) seedling production systems were evaluated and compared with traditional systems to identify the effects of new techniques.

Design/Methodology/Approach: Five chili varieties from different regions were evaluated (S1O, S2B, S3L, S4J, and S5A). The methods used in the experiment were greenhouse germination trays (GT) and open field nurseries (OFN). In addition, traditional (T), conventional (C), and alternative (A) fertilization systems were used. The variables evaluated were plant height (PH), stem diameter (SD), and number of leaves (NL).

Results: The GT+A fertilization treatment increased the PH and NL of the seedlings. The OFN+T combination stood out during the last stages, recording a higher PH. Meanwhile, the GT+C seedlings achieved a regular growth, obtaining good ST and NL results during the intermediate stages. At 30 and 45 days, S2B+GT+T recorded the highest PH and NL, while, at 60 days, S2B+GT+A obtained the highest SD and PH. At 90 days, S2B+OFN+T recorded the highest PH.

Study Limitations/Implications: The seedling production of the open field nurseries was 15 days behind the 60-day greenhouse germination tray system (GT) with peat moss.

Findings/Conclusions: The traditional method recorded the most homogenous plants. The OFN+A treatment and the OFN+T treatment had good performance and adaptability. The root analysis showed that the A system promoted a better root development. Management systems did not impact the health quality of the crops.

Keywords: Chili crops, *Capsicum annuum* L., alternative, conventional, and traditional agriculture.

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INTRODUCTION

The cultural and natural richness of Mexico has enabled the development to highly valued products worldwide. These products are protected under the Designation of Origin (DO) standard. Currently, the country has 18 DO certificates, granted by the Instituto Mexicano de la Propiedad Industrial (IMPI) (Villaseñor, 2020). The Yahualica chile de



árbol stands out among these products. Its DO certification was granted in 2018 to 11 municipalities: nine in Jalisco and two in Zacatecas (Economía, 2018). The DO certificate guarantees the authenticity and origin of this chili, protecting traditional techniques and strengthening the cultural identity of the producer communities (SIAP, 2024).

Chile de árbol production is fundamental for the local economy in the Altos Norte region of Jalisco and the southern region of Zacatecas, providing income for 400 producers, who produce 1,440 t in \approx 1,200 ha every year (Saavedra, 2022). However, this crop faces challenges, including phytosanitary issues and adverse climate conditions, that impact the first stages of its development, particularly, during the seedling production stage (SIAP, 2021).

The quality of the seedlings is fundamental for a successful chili production, because it directly influences crop yield and resistance to diseases and stress conditions (Martínez *et al.*, 2019). Height, stem diameter, number of leaves, root development, and plant health are some of the characteristics that define a good quality seedling (López-Pérez *et al.*, 2020). A vigorous and healthy seedling is easier to establish in the field, maximizing productive potential (Gómez *et al.*, 2018).

Seedling production systems include open field nurseries (OFN) and greenhouse germination trays (GT). Each system has specific costs, management, and quality advantages and challenges (Rodríguez *et al.*, 2021). Adopting modern technologies and sustainable agricultural practices can significantly improve traditional systems and, consequently, the quality of Yahualica *chile de árbol* seedlings, increasing its competitiveness in the market (Hernández and Ramírez, 2022). Therefore, different seedling production systems were evaluated in order to identify the effect of the implementation of new technologies and to compare them with the traditional system. The objective was to optimize the quality, innocuousness, and yield of *chile de árbol* crops, guaranteeing their long-term sustainability in a region with great cultural and economic importance.

MATERIALS AND METHODS

Location of the Study Area

The research was conducted from January to May 2024, in the facilities of Invernaderos Icuata, located 3 km away from the municipal seat of Nochistlán de Mejía, Zacatecas, México (21° 21' 23" N and 102° 52' 30" W, at 1,899 m.a.s.l.) (Figure 1) (INEGI, 2020). The area has a monsoon influenced humid subtropical climate (Cwa), with a 14-20 °C annual temperature and a 700-1,000 mm annual rainfall (García, 2004). The main soils of the region are phaeozem and regosol (INEGI, 2018).

Plant Material

This research evaluated the performance of five varieties of *chile de árbol* seeds in three production systems: traditional (T), conventional (C), and alternative (A). The seeds came from municipalities within the designation of origin region. These systems were established based on: (a) sowing system, consisting of germination trays (GT) with substrate in a protected environment and open field nurseries (OFN); and (b) source of the fertilizer. Seeds from five varieties of *chile de árbol* were extracted from dry mature



Figure 1. Location of the study area.

fruits. One of the five varieties was an endemic regional plant, known as the “original plant.” This variety has been preserved in backyards. The other four seed varieties are native to the region and were collected in three communities by local producers (Table 1): Benjamín Santos de La Portilla, Nochistlán; Leandro Yáñez, Apulco, Apulco; José Sandoval, El Salto Verde, and Yahualica, and Luis Antonio Plascencia, Manalisco, Yahualica. The seeds were identified as S1O, S2B, S3L, S4J, and S5A, respectively. All the seeds were treated with 10% trisodium phosphate to reduce their seedborne pathogen load (Díaz *et al.*, 2019).

Experimental Design

Two sowing patterns were used for each type of seed: greenhouse germination trays (GT) and open field nurseries (OFN). Three fertilization arrangements were applied: traditional (T), conventional (C), and alternative (A) (Table 2). The experiment was developed using a randomized complete block design, with three repetitions per treatment. The experimental units in the greenhouse consisted of 15 rows with three

Table 1. Description of the *chile de árbol* factors evaluated.

Factors	Level	Identifier
Seed type of sowing	Original	S1O
	Benjamín	S2B
	Leandro	S3L
	José	S4J
	Antonio	S5A
fertilization system	Tray	Ch
	Seed bed	Al
	Traditional	T
	Conventional	C
	Alternative	A

Table 2. Fertilization arrangements of *Yahualica chile de árbol* seedlings in different systems.

System	Product	Content
Traditional	Ammonium sulfate (NH ₄) ₂ SO ₄	21-0-0-24S
Conventional	Various sources	38-52-80-16S
	Micronutrients	0.1%B, 0.05%Cu, 0.1%Fe, 0.05%Mn, 0.01%Mo, 0.1%Zn
Alternative	<i>Bacillus subtilis</i>	1×10 ¹⁰ UFC/mL

columns of 200 cavity trays, established 0.1 m apart from each other. The open field seeds were sown in nine rows, with four columns, in 1×1.20 m nurseries, 0.50 m apart from each other.

Sowing and Management

The germination trays were filled with a 75% peat and 25% coconut fiber substrate. One seed was sown per cavity. The seeds were irrigated at field capacity, covered with a black plastic, and stored in a warehouse for 10 days. After the seeds sprouted, they were placed inside a greenhouse with a 720-caliper polyethylene cover, above a 0.65 m base made up of plastic washbasins. The seedlings were sprinkled every day and each tray received 1 L of water.

Meanwhile, the nurseries were established at ground level. Dry cow manure was burnt on top of the soil (cow horn manure method), following the traditional fertilization method and using 19 L of water. Sowing was conducted above the ashes.

Before the sowing, the alternative system was treated with a liquid inoculant with 99.98 % mycorrhizae (*Bacillus subtilis*) (8×10¹⁰ spore/mL of water). In the conventional system, the seeds were directly sown in the soil. Two-hundred and fifty seeds were used in all the systems. All the nurseries were covered with a 720-caliper polyethylene cover for 22 days. After the seeds sprouted, they were only covered during the night. The cover was completely removed approximately 45 days after the sowing.

After their germination, seedlings were irrigated every third day, using the waterlogging method. Fertilization was conducted 20 days after the sowing and, subsequently, it was performed weekly in both sowing patterns, depending on each system. For its part, the traditional system was focused on a rich N and S source. As a result of the application of the 30% nutritive solution proposed by Hewit and Smith (1952) and modified by Gómez *et al.* (2019), the N, P, K, and S content in the soil of the conventional system was more balanced. Finally, 1×10¹⁰ UFC/mL *Bacillus subtilis* was used for the soils of the alternative system, in order to release its nutrients (Table 2).

Response Variables

Five seedlings from each nursery and five seedlings from each tray were randomly selected and subjected to a growth analysis. The measurements were carried out at 30, 45, 60, and 90 days after sowing.

Plant Height (PH - cm): measured from the base of the stem to the apex of the seedling, with a flexometer (cm/mm), following the recommendations of López *et al.* (2018).

Stem Diameter (SD - mm): measured in the center of the stem, between the soil or substrate and the first knot, with a Vernier or a caliper (mm), following the recommendations of González and Pérez (2019).

Number of Leaves (NL): every pair of leaves was counted. The leaves located after the knot were considered as a single leaf, following the recommendations of Hernández (2020).

Root Length (RL - cm): measured from the base of the stem to the point of the main root, with a 50×50 cm horizontally and vertically graduated wood board (Ramírez *et al.*, 2017).

Root Volume (RV - ml): determined using the water displacement method, in a 100 mL measuring cylinder. The whole root was immersed and the displaced water volume was measured, following the recommendations of López and Sánchez (2019).

Rot Health (RH): evaluated through the observation of the color of the root. Completely white roots were considered healthy, while brownish or yellowish roots were classified as sick (Hernández *et al.* 2020).

Statistical Analysis

The ST, PH, and NL response variables were subjected to an analysis of variance (ANOVA) to establish differences that could have been influenced by the following factors: origin of the seed, sowing type, and fertilization system. In addition, Tukey Honestly Significant Difference (HSD) ($P \leq 0.05$) was used to compare and evaluate the differences between treatments. Both evaluations were conducted on sampling days 30, 45, 60, and 90. RL and RV were subjected to an analysis of variance (ANOVA), including interactions between the seed, sowing type, and fertilization system factors. This evaluation was conducted to establish their impact on the development of the root. In addition, the results were compared using the Tukey's method for multiple comparisons. Contrasts were applied to compare fertilizations systems. Meanwhile, the normality of the residuals was verified through statistical tests and graphs. Finally, boxplots were developed to visualize the distribution and the correlation between length and volume was calculated. The analysis was carried out with the SAS Studio v.3.81 software.

RESULTS AND DISCUSSION

Plant Height (PH)

The model was significant during sampling days 30, 45, and 60. However, no significant effects were recorded during day 90. This situation suggested that the differences between treatments were sharper during the intermediate stages (30-60 days) (Table 3).

Differences ($P \leq 0.05$) were recorded in the average PH between treatments. On day 30, S2B+GT+T recorded the highest development (2.5 cm), followed by S3L+GT+C (2.1 cm). On day 45, S2B+OFN+T obtained the highest result (3.0 cm), followed by S4J+OFN+A (2.8 cm). These results showed the first differences between sowing systems. On day 60, S2B+GT+T stood out again (3.5 cm), but all the combinations recorded an overall PH increase.

Meanwhile, on day 90, S2B+OFN+T still recorded the highest results (4.0 cm), followed by S4J+OFN+A (3.9 cm), as the difference between treatments stabilized (Figure 2). Over

Table 3. Factors and interactions that had a significant impact on the height of *chile de árbol* seedling during each sampling.

Sampling	Effects Significant	Interactions	Combination Outstanding
Día 30	Seed (p<0.05) System (p<0.01)	Variety×System (p=0.0039)	S2B+Ch+T
Día 45	Seed (p<0.01) System (p<0.05) Type (p<0.05)	Variety×System (p<0.0001) Variety×Type (p<0.0001)	S2B+Al+T
Día 60	System (p<0.01) Seed (p<0.01)	Variety×System×Type (p=0.0311)	S4J+Ch+A
Día 90	Seed (p=0.5402)	Variety×System (not significant)	S2B+Al+T

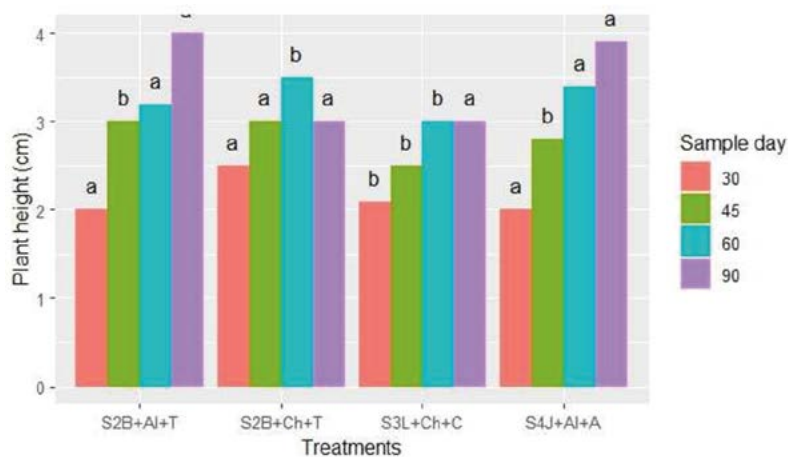


Figure 2. Evolution of the height of *chile de árbol* seedlings at 30 and 90 days after the sowing in treatments with the best performance.

time, the alternative system (A) favored PH growth, particularly during the intermediate stages (45 and 60 days), while the traditional system (T) recorded the lowest growth in all the stages, especially during the first days of the experiment. Meanwhile, the conventional system (C) showed a medium growth, particularly during the final stages. Regarding sowing type, GT favored a faster growth during the first stages (30 and 45 days), while OFN recorded a more competitive growth during the last stages (60 and 90 days), showing a greater PH during the last measurements.

Stem Diameter (SD)

Significant SD differences were recorded on sampling days 30, 45, and 60. On day 90, seed had a significant effect; however, no significant interactions were found. The SD differences were more noticeable during the early development stages; however, they stabilized towards the end of the growth cycle (Table 4).

Significant differences were recorded between treatments; S2B stood out with the highest ST average in all the stages. On day 30, S2B+GT+T recorded the highest ST (2.10 mm), while S3L+GT+C obtained the lowest ST (1.80 mm). On day 45, S2B+OFN+T

Table 4. Factors and interactions that had a significant impact on the stem diameter of *chile de árbol* seedling during each sampling.

Sampling (day)	Effect Significant	Interaction	Outstanding
30	Seed (p<0.05) System (p<0.01)	Seed×System (p<0.01)	S2B+Ch+T
45	Seed (p<0.01), System (p<0.05) Type (p<0.05)	Seed×System (p<0.0001) Seed×Type (p<0.0001)	S2B+Al+T
Da 60	System (p<0.01) Seed (p<0.01)	Seed×System×Type (p=0.0311)	S2B+Ch+A
90	Seed (p=0.01)	Seed×System (not significant)	S2B+Al+T

and S2B+OFN+A obtained the highest ST (2.5 mm), followed by S4J+OFN+A (2.40 mm). On day 60, the ST was more homogenous, with S2B+GT+T standing out once again (2.80 mm).

On day 90, the differences stabilized, with S2B+OFN+T reaching a 3.00 mm SD and showing a steady growth (Figure 3). The traditional (T) and alternative (A) systems produced higher ST than the conventional (C) system in all stages. OFN recorded a higher ST during the final stages, while GT was more competitive during the intermediate stages. These results highlighted the significant influence of the factors and their interactions in the development of the SD.

Number of Leaves (NL)

The results of the ANOVA indicated that this variable had an impact (p<0.05) during all sampling days, except for day 90. The results suggest that NL was influenced by the variety, sowing type, and fertilization system factors, recording significant differences throughout the experiment (Table 5).

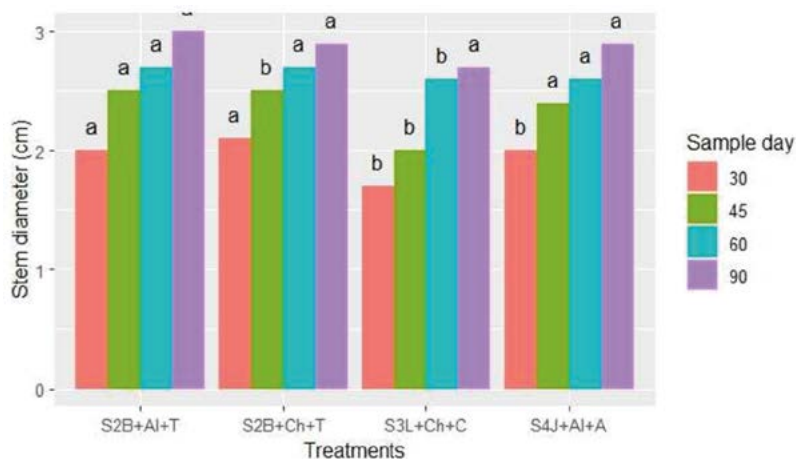


Figure 3. Evolution of the stem diameter of *chile de árbol* seedlings at 30 and 90 days after the sowing in the treatments with the best performance.

Table 5. Factors and interactions that had a significant impact on the number of leaves of *chile de árbol* seedling during each sampling.

Sampling (day)	Effect Significant	Interaction	Combination Outstanding
30	Seed (p<0.05) System (p<0.01)	Seed×System (p<0.01)	S2B+Ch+T
45	Seed (p<0.01) System (p<0.05) Type (p<0.05)	Seed×System (p<0.0001) Seed×Type (p<0.0001)	S2B+Al+T
60	System (p<0.01) Seed (p<0.01)	Seed×System×Tipo (p=0.0311)	S4J+Ch+T
90	System (p=0.305)	Seed×System (not significant)	S2B+Al+T

On day 30, S2B+GT+T recorded the highest NL average (6.8). The differences were particularly marked between fertilization systems. On day 45, S2B+OFN+T reached the highest growth (8.5 leaves), followed by S3L+GT+C (7.2 leaves), recording major differences between sowing types. On day 60, S4J+OFN+A obtained the highest NL (10.2 leaves), followed by S3L+GT+A (9.1 leaves). These results indicated an overall NL increase in all the treatments. Finally, on day 90, S2B+OFN+T reached the highest yield (12 leaves), followed by S4J+OFN+A (11.8 leaves) (Figure 4).

Over time, the alternative system (A) showed a steeper NL, particularly during the intermediate stages (45 and 60 days). The traditional system (T) recorded the lowest NL during all the sampling stages, with a slight increase towards the end. Meanwhile, the conventional system (C) had a medium performance, although it stood out during the final stages (60 and 90 days). Regarding sowing type, GT favored a higher NL during the first stages (30 and 45 days), while OFN was more effective during the final stages (60 and 90 days).

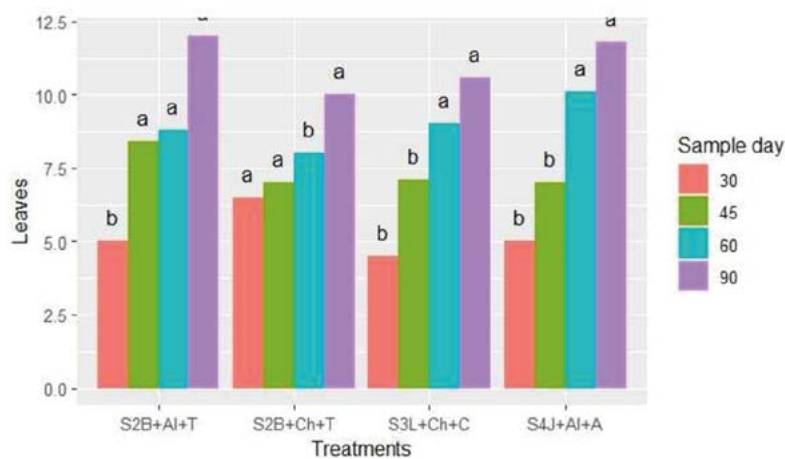


Figure 4. Evolution of the number of leaves of *chile de árbol* seedlings at 30 and 90 days after the sowing in the treatments with the best performance.

Root Length

The GLM analysis for the root length indicated that the model was highly significant, recording a high F-value and a <0.0001 p-value. These results suggest that the explicative variables significantly impacted root length.

The root length contrast between the traditional and conventional systems and the alternative system recorded a significant difference: a high F-value and a <0.0001 p-value. These results suggest that the traditional system had a different effect in root length than the other two systems. The length normality test of the residuals suggested that they were normally distributed. Clear mean differences were recorded between the systems (Figure 5), indicating that some production systems resulted in longer roots than the other systems. Possible atypical values suggest a variability within the systems.

Root Volume

The GLM model also showed a significant root volume, recording a high F-value and a <0.0001 p-value. These results indicate the strong influence of the variables in root volume. The root volume contrast between the traditional and the conventional system *vs.* the alternative system had significant differences: a high F-value and a <0.0001 p-value.

The normality test of the residuals suggested that they were normally distributed, strengthening the validity of the model. Furthermore, the length-volume correlation showed a significant positive ratio: root volume increased along with root length.

Just like in the case of length, differences were recorded regarding mean and data dispersion. In average, some systems resulted in a larger root volume (Figure 6). Atypical values were also clear, reflecting the response variability of root volume within the systems.

Root Health

All the evaluated seedlings had a white or cream and firm and homogeneous roots. This situation suggests that all the roots were healthy in all the treatments.

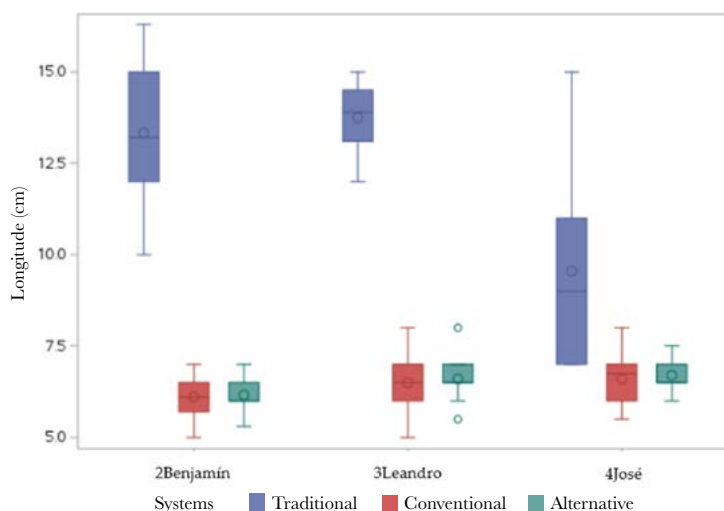


Figure 5. Root length comparison of different *chile de árbol* seeds grown under different systems.

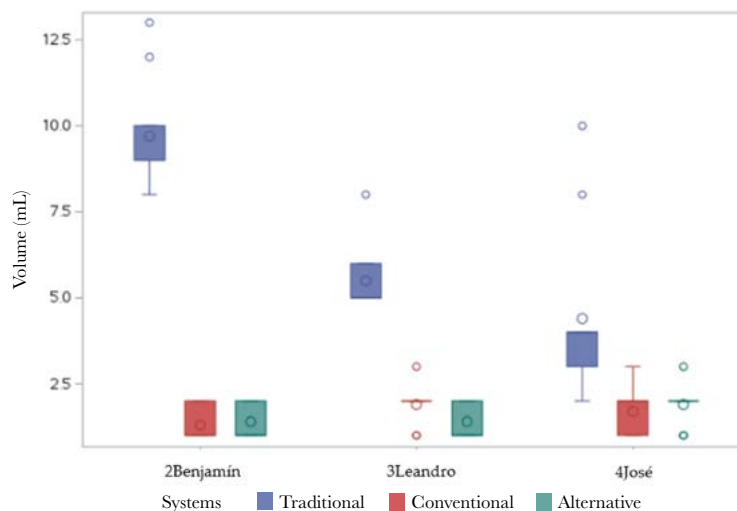


Figure 6. Comparison of root volume of different *chile de árbol* seeds grown under different systems.

The interaction between the genetic material effect and the agronomic management significantly influenced the growth of chili seedlings. The S2B seed stood out in PH, SD, and NL under the traditional fertilization system. For its part, S4J stood out during the final stages and in less conventional systems (*e.g.*, OFN+A), while S3L had a limited performance in most scenarios.

According to Bosland and Votava (2012), this response shows the genetic capacity to efficiently use nutrients and genetic plasticity to adapt to changeable environments. Selecting genotypes adapted to specific sowing and fertilization practices optimizes the genetic-environment interaction, promoting a more consistent and strong crop development (Rodríguez-Burruezo and Prohens, 2005). Meanwhile, the performance of the seeds could have been impacted by factors out of the control of the research team, such as the method used to dry them. Moo-Muñoz *et al.* (2016) pointed out that the drying method could impact the characteristics and growth of seedlings.

Based on Bautista and Sánchez (2017), the results of this study showed that, depending on the growing stage of the crop, the GT and OFN systems had a distinguished influence on the growth of chili the árbol seedlings. During the first stages, GT favored a vigorous initial growth thanks to the controlled conditions of the greenhouse that optimized water and nutrient availability. Such was the case of S2B+GT+T, which recorded the highest PH, SD, and NL at day 30. In contrast, OFN showed significant advantages during the final stages, particularly S2B+OFN+T and S4J+OFN+A, at day 90.

Meanwhile, environmental conditions significantly influenced the growth of chili seedlings, particularly SD and NL (Medina-Lara and Martínez-Damián, 2020). During the initial stages, the controlled conditions and protected environment of the GT system favored a homogenous growth: S2B stood out with a 2.1 mm SD and 6.8 leaves at day 30. Nevertheless, during the final stages, the OFN system promoted a stronger structural development: S2B+OFN+T reached a 3.00 mm diameter and had 12 leaves at day 90.

Meanwhile, the high temperatures within the greenhouse during certain days ($>50\text{ }^{\circ}\text{C}$) reduced the growth of the GT seedlings. These results match the findings of Rosmaina *et al.* (2021), who pointed out that chili seedlings subjected to high temperatures ($\geq 35\text{ }^{\circ}\text{C}$) reduced both crown and root growth.

Fertilization System

The results indicated that the traditional fertilization system was more effective for the growth of chili seedlings, particularly during the initial and intermediate stages. This system—based on local practices and the balanced management, N, P, K, and other nutrients—favored a homogenous early growth and a strong structural development (Ochoa-Velasco and Cruz-Valenzuela 2016). For example, S2B recorded the highest PH, SD, and NL, at 30 and 60 days. The impact of the alternative fertilization system showed more variability; however, the seedlings showed a good development during the final stages (genotype 4J). These results match the findings of Castillo-Aguilar *et al.* (2017), who reported similar data for *Capsicum chinense* Jacq. seeds, inoculated with rhizobacteria that positively impacted PH and SD growth. In addition, Alori *et al.* (2017) pointed out that the use of microbial inoculum improves the soil capacity to provide N, P, and K to the crops, favoring soil structural stability and mitigating plant stress, caused by draught, soil pollution, and salinity.

These findings highlight the importance of combining traditional practices with complementary approaches, adjusting them to genotype and specific crop stages, in order to maximize chili plant growth throughout their development cycle. Organic fertilization in both the alternative and traditional systems improved soil quality and favored a sustainable growth; however, this type of fertilization took longer to provide nutrients, impacting the yield of certain genotypes. For its part, the chemical fertilization used in the conventional system drove initial growth, but it was less sustainable (Sánchez and Osuna, 2019).

Production System

A production system is a set of agricultural practices applied to the crop, such as sowing methods, soil management, irrigation, fertilization, and pest control. The results obtained in this study highlight the importance of these production systems in the development of roots, particularly root length and volume. These findings were consistent with the reports of Smith *et al.* (2020), who pointed out that production systems have a significant influence on the morphological characteristics of roots, impacting both their length and volume. The adoption of alternative systems has shown different effects, suggesting that crop management optimization could improve root development.

CONCLUSIONS

Benjamín seed (S2B)+GT+nutrient intake, under the traditional system, was the most efficient combination during the initial stage, recording the highest PH, SD, and NL. Their growth was driven by the protected environmental conditions and the nutrient intake within the greenhouse. During the final stages, the Benjamín (S2B)+OFN+Traditional combination stood out, showing the strongest and homogenous development. In addition,

José (S4J)+OFN+Alternative and Leandro (S3L)+OFN+Traditional had an outstanding adaptability to open field conditions and to sustainable fertilization systems during the final stages of the crop. Meanwhile, the alternative system promoted a better root development. The positive root length-volume correlation highlighted a direct ratio between both parameters. Finally, the optimal health of all the treatments showed that neither system impacted the health quality of the crops.

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