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Optimizing Tomato Seedling Production in the Tropics: Effects of Trichoderma, Arbuscular Mycorrhizal Fungi, and Key Agronomical Factors

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Abstract: Agriculture remains a key contributor to Central America's economy, despite climate change posing a significant threat to the sector. In the Trifinio region, already afflicted by arid summers, temperatures are expected to rise in the near future, potentially exacerbating the vulnerability of smallholder farmers. This study investigates the effects of two fungal symbionts, *Trichoderma asperellum* (TR) and the Arbuscular mycorrhiza fungi (AMF) *Glomus cubense*, and agronomic choices and practices such as cultivar selection, substrate type, and fertigation management on tomato (*Solanum lycopersicum* L.) seedling growth and quality. Results showed that nutrient solution and the adoption of forest topsoil as substrate significantly enhanced morphological, physiological, and quality parameters. Modifying the nutrient solution to allow for an increase in plant height of 170% and a dry weight of 163% and enhancing Dickson's quality index (DQI) by 64.5%, while the use of forest topsoil resulted in plants 58.6% higher, with an increase of 101% in dry weight and of 90.1% in the DQI. Both *T. asperellum* and *G. cubense* had positive effects on specific growth parameters; for instance, TR increased leaf number (+6.95%), while AMF increased stem diameter (+3.56%) and root length (+19.1%), although they did not, overall, significantly increase the seedling's biomass and quality. These findings underscore the importance of agronomic practices in mitigating the impacts of climate change on tomato production, offering valuable insights for farmers in semi-arid regions.

Keywords: substrates; fertigation; cultivar; nursery management; horticultural crops

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1. Introduction

The horticultural sector in Central America mostly exports vegetables, fruits, nuts, coffee, and cocoa, which enables generating a positive net agricultural trade balance. Despite the decreased relative contribution of agriculture to the economy due to the growth of other sectors, it remains crucial, significantly contributing to countries' gross domestic

product [1]. Conversely, the agricultural production and the numerous livelihoods dependent on it are threatened by rapidly changing climate conditions. Countries such as El Salvador, Guatemala, and Honduras are among the most affected by extreme weather events in the Americas, ranking 28th, 16th, and 44th, respectively, in the Global Climate Risk Index [2]. Currently, approximately 50% of smallholder farmers in the Trifinio region live below the poverty line [3], and climate change is likely to exacerbate their situation by reducing suitable farmland [4,5]. In El Salvador, tomato (*Solanum lycopersicum* L.) is one of the most cultivated horticultural products, with 23,359 tonnes produced in 2020 [6]. Along its cultivation, the early seedling stage is a crucial phase that determines plant growth and yield and is sensitive to abiotic stresses, such as salinity [7,8] and temperatures [9], as well as biotic stresses such as seed-borne fungal infections [10].

As temperatures rise, tomato production is expected to decrease, while more irrigation water will be needed to sustain yields [11]. Addressing the impacts of climate change on agricultural production requires a range of solutions, from traditional practices like selecting optimal agronomic strategies to more innovative approaches that foster beneficial symbioses. Arbuscular mycorrhiza fungi (AMF), for instance, establish beneficial interactions with plants, enhancing nutrient uptake, accelerating root development [12], and activating drought tolerance mechanisms [13]. *Glomus cubense* is an AMF firstly isolated in Cuba [14] and now commercially available in Central America. The beneficial effects of *G. cubense* on plant growth have been demonstrated both on peanut through the seed coating technique [15] and on tomato through the application of AMF-enriched liquid biofertilizer [16]. Another group of fungi extensively studied as plant growth promoters and biocontrol agents includes the opportunistic, avirulent plant symbionts *Trichoderma* spp. [17–19]. Their mode of action can be indirect, by competing for space and resources with other fungi and activating plant defense mechanisms, or direct, by producing active metabolites that inhibit pathogenic fungi growth as found in tomato [20]. Some *Trichoderma* spp. isolates have also been found to reduce water-deficit stress and increase root volume [21]. While both *Trichoderma* spp. and *G. cubense* have been tested individually, there is no evidence regarding the effect of their interaction on plant growth and how they interact with other relevant agronomical factors. Moreover, most of the studies conducted in the past are focusing on temperate regions, while evidence on tropical and semi-arid areas is lacking [22]. This could be particularly relevant for the farmers of the Trifinio region, where nursery practices are not optimized. For instance, although it is well known that fertigation improves the growth of seedlings [23,24], farmers in the region still do not fertigate transplants even when nutrient content in the substrate is rather low, as in peat substrates. Transplants can be grown in peat-based substrates or in a variety of other substrates [25–28] depending on their availability, but some of them have a nutrient content that may allow for reduced N application, which has to be taken into account in the fertilization program.

In this study we assessed the effect of *Trichoderma asperellum* strain T-90 and AMF *Glomus cubense* on the growth of tomato seedlings under natural semi-arid conditions. Additionally, we tested the effects of other agronomical factors influencing the initial development phase, i.e., tomato cultivar, growing substrate, and fertigation, to reveal possible interactions with the fungal symbiosis and seedling's growth. The aim of the research is to identify relevant factors that small-scale farmers can easily implement in the area to produce quality tomato seedlings despite the harsh climatic conditions.

2. Materials and Methods

2.1. Location and Climatic Conditions

The experiment took place in an open field in the Technological Innovation Centre SISTAGRO, located in Metapán, in the department of Santa Ana, El Salvador (14°33′ North,

−89°45′ Est, 477 m above sea level). According to the Köppen classification, the local climate is *Aw* type, tropical savannah with a dry summer and a rainy season concentrated between June and October [29,30]. Climatic parameters at bench level during the experiment (temperature and relative humidity) were measured daily using a digital thermometer with a resolution of 0.1 °C (Thermo Fisher Scientific, Waltham, MA, USA).

2.2. Treatments and Experimental Design

The experimental design was a randomized block design with five treatment variables and three replicates. The five treatment variables, each with two levels, were 1—tomato cultivar: open-pollinated Valiente (VAL) or hybrid Pony (PON); 2—substrate: peat (PEAT) or forest topsoil (US); 3—fertigation: untreated (irrigation with plain water [WA]) or treated (irrigated with nutrient solution [NS]); 4—*Trichoderma* application: not treated (NTR) or treated (TR); and 5—arbuscular mycorrhiza fungi (AMF) application: not treated (NM) or treated (M). The details of the experimental design are also summarized in the Supplementary Materials (Table S1 and Figure S1). The factorial experimental design accounted for 32 treatments (2 Cv × 2 Sub × 2 Fertig × 2 *Trichoderma* × 2 AMF). Each cultivation tray hosted 128 seedlings belonging to the 2 cultivars (64 plants per cultivar), and since each treatment was replicated in three different trays, it accounted for 192 seedlings.

Cultivar. Tomato (*Solanum lycopersicum* L.) cultivars were selected among those locally available and commonly adopted by growers, namely an open-pollinated cultivar named Valiente (VAL) (AMER CONSULTORES S.A. de C.V, Lourdes Colon, El Salvador) and a hybrid cultivar named Pony (El Surco S.A de C.V., La Libertad, El Salvador).

Substrate. The peat substrate was namely Canadian sphagnum peat, composed of 80% sphagnum peat, perlite, calcitic limestone, dolomitic limestone, and a wetting agent. Its pH ranged between 5.4 and 6.3, with an electrical conductivity (EC) of 1000–1500 µS/cm. The forest topsoil (US) was collected from the wooded areas of Chalatenango, La Palma (El Salvador), and featured the following composition: 1.2% N, 0.12% P, and 0.52% K. Substrates were physically and chemically characterized, and results are reported in Table 1.

Table 1. Physical and chemical characteristics of the substrate evaluated (n = 3).

Parameter	Unit	Methodology	Peat	Forest Soil
Bulk density	g/L	EN 13040 (1999)	240.6 ± 27.1	370.5 ± 34.6
Total pore space	%	NCSU porometer *	76.8 ± 3.9	79.4 ± 3.0
Air-filled porosity	%	NCSU porometer	36.0 ± 3.2	54.2 ± 3.5
Water holding capacity	%	NCSU porometer	40.8 ± 0.9	25.2 ± 1.8
pH		EN 13037 (1999)	6.17 ± 0.1	7.87 ± 0.1
Electrical conductivity	mS/cm	EN 13038 (1999)	1.34 ± 0.1	3.32 ± 0.7

* [31].

Fertigation. For irrigation, the local water (WA) was used, which had an electrical conductivity (EC) of 580 µS/cm and a pH of 6.5. The nutrient solution (NS) used was prepared by adding 0.7 g/L soluble fertilizer, up to an EC of about 1200 µS/cm. The fertilizer used was *Solo Feed Inicio* (Duwest, Guatemala City, Guatemala) and was composed as follows: N 19.26% (13.51% Urea-N, 5.75% Ammoniacal-N); P_2O_5 28.44%; K_2O 9.61%; Mg 0.45%; S 3.83%; inert ingredients 38.41%. Irrigation with WA or NS started 8 days after germination (e.g., 8 days after sowing) and was supplied three times a day, at 8 a.m., 11 a.m., and 3 p.m., with a volume of 0.75 L per tray per application, for a total daily application of 2.25 L per tray.

Trichoderma. The selected strain was *Trichoderma asperellum* T-90, applied using the product BioTRICH T-90 (concentration of >10^8 conidia per gram of product) (BioAmigo, La Palma, El Salvador). For each tray, 10 g of commercial formulation was added to 8 L of substrate, which was then brought to field capacity with water and consequentially used to fill the trays. The inoculation was performed five days before planting to allow substrate colonization. During this period, trays were covered with natural straw to preserve moisture and facilitate fungal development. According to the supplier's instruction, after seeds' germination, a solution of 3.75 mg/L of the commercial product BioTRICH, prepared by diluting 30 g of product into 8 L of water, was applied using a sprayer. The application occurred at 5:00 p.m. in two events, specifically at 13 and 18 days after sowing.

Arbuscular mycorrhiza fungi (AMF). The AMF *Globus cubense* was applied using the product Biofertilizer Bioamigo (composition of >20 spores per gram of product) (BioAmigo, La Palma, El Salvador). The treated seeds were uniformly coated with the commercial formulation and allowed to dry in a shaded area for fifteen minutes before sowing.

2.3. Crop Management

The experiment took place between 8 April and 29 April 2024. Sowing was conducted manually on April 8 on plastic trays (54 × 28 × 5 cm) featuring 128 cells. The seedling trays were placed in an outdoor nursery, covered by a shade net that resulted in a 63% solar light reduction. During the five days following sowing, the trays were watered using only water three times a day (at 8 a.m., 11 a.m., and 3 p.m.) using a watering can. When 80% of the seedlings had fully expanded cotyledons, the germination process was considered complete, and the treatment with NS started. At 21 days after sowing, seedlings were harvested and parameters measured immediately. Measures were performed on 4 seedlings per replication, which leads to 12 seedlings per treatment.

2.4. Biometric Parameters

Plant height and number of leaves. The height of the seedlings was measured using a standard ruler with a precision of 1 mm. Measurements were taken from the plant collar to the tip of the highest leaf. The number of leaves of the plant was then counted, excluding cotyledons.

Stem diameter. Measurements of stem diameters were taken in the portion of the stem from collar to cotyledons using a hand caliper with a precision of 0.05 mm.

Root length. Roots were firstly washed with water to remove any adhering particles. Then measurement was taken using a ruler with 0.1 mm precision, from the root collar to the tip of the longest root.

2.5. Dry Matter Yield and Partitioning

Fresh weight, dry weight (DW), and dry matter percentage. Seedlings were separated into roots and shoots, and fresh weight was recorded, respectively, using a precision scale. Then, they were placed in a laboratory oven at 70 °C for 72 h for dehydration. Completed the drying process, shoot and root DW were measured. Total fresh (TFW) and dry weight (TDW) were calculated as the sum of shoot and root fresh and dry weights. Dry matter content was calculated as follows:

$$Dry\ matter\ (\%) = TDW/TFW \times 100 \quad (1)$$

Dry matter partition. The dry matter partitioning was determined as follows:

$$Partitioning\ in\ Shoot\ (\%) = DM_{shoot}/TDW \times 100 \quad (2)$$

$$Partitioning\ in\ Root\ (\%) = DM_{root}/TDW \times 100 \quad (3)$$

2.6. Leaf Temperature

Leaf temperature was measured on the apical leaf between 12 pm and 2 pm. This specific time frame was selected as it corresponded to the daily peak solar radiation and ambient temperature. Leaf temperature was measured using an infrared thermometer, 62 MAX+ (Fluke®, Washington, DC, USA), with an optical resolution of 12:1.

2.7. Quality Parameters

Stem diameter ratio. As detailed in the previous section, the stem diameter was measured between collar and cotyledons. A second diameter was measured between the cotyledons and the first true leaf. The stem diameter ratio was calculated by dividing this second diameter by the first one.

Sturdiness quotient. The sturdiness quotient (SQ) was calculated as the ratio between the stem diameter and the seedling height [32].

Root dry weight/Root length ratio. The root dry weight/root length (RDW/RL) ratio is a physiological parameter that reflects plant quality [33].

Quality index. The quality index was calculated in accordance with Dickson et al. [32]. The DQI formula is described as follows:

$$DQI = TDW/\left(\frac{SH}{SD} + \frac{SDW}{RDW}\right) \quad (4)$$

where DQI = Dickson quality index, TDW = total dry weight (g), SH = seedling height (cm), SD = stem diameter (mm), SDW = shoot dry weight (g), and RDW = root dry weight (g).

2.8. Climatic Parameters

Overall, the climate recorded was hot and dry (Figure 1). During the experiment, average daily temperatures ranged from 25.6 °C to 36.4 °C. Maximum temperature reached up to 44.5 °C on the day of sowing. Relative humidity (RH) was generally low, maintaining values between 20 and 60%. Only on 9 April, RH reached 90% due to a rainy event.

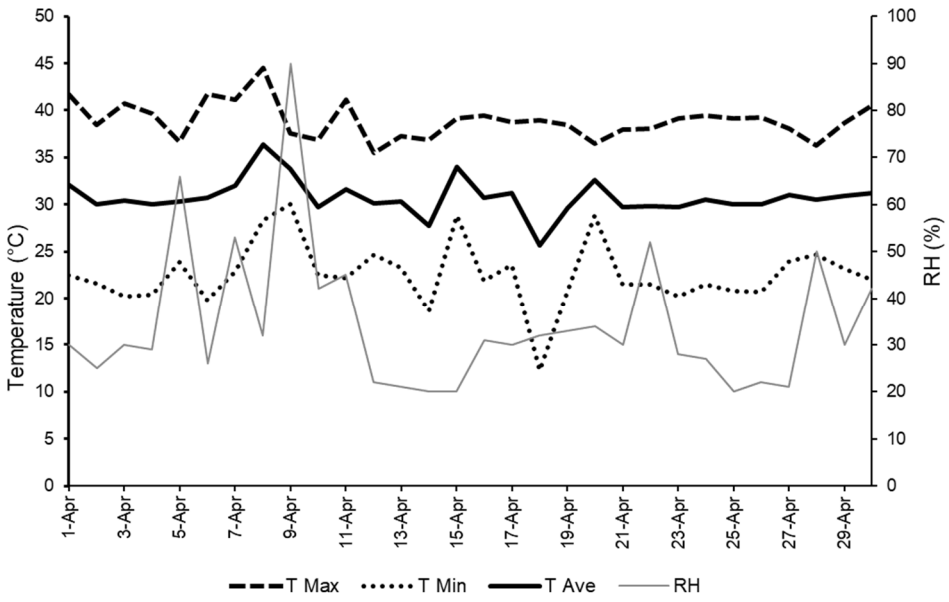


Figure 1. Climatic parameters recorded from the start (S) to the end (E) of the experiment. Black lines represent the daily temperatures (T), while the grey line indicates the relative humidity (RH).

2.9. Statistical Analysis

As indicated above, most parameters were measured on 4 seedlings per replication, which leads to 12 seedlings per treatment. This is with the exception of the fresh and dry weight (and consequently derived values, e.g., dry matter percentage, dry matter partitioning, Dickson quality index), which were calculated on 3 seedlings per replication, i.e., 9 per treatment.

Resulting data were subjected to analysis of variance (ANOVA). Means were separated using Tukey's honestly significant difference test (HSD) at $p \leq 0.01$. Before the analysis, all data were checked for normality through the Shapiro-Wilk test and homogeneity of variance through Bartlett's test. Statistical analyses were carried out using software R (version 4.3.0) [34] using the "emmeans" [35], "dplyr" [36], and "multcomp" [37] packages. Due to the number of comparisons performed and to present only robust, statistically significant findings, only significant differences for $p \leq 0.01$ and significant first-order interactions are hereby presented and discussed.

In addition to ANOVA, a principal component analysis (PCA) was conducted to explore the multivariate relationships among the measured variables and identify underlying patterns or clusters within the dataset. To run the analysis, the R environment (RStudio, Version: 2024.12.0 + 467 by Posit (Boston, MA, USA), and R software, Version: 4.3.3, by the R Foundation for Statistical Computing) was used.

3. Results

3.1. Responses of Biometric Parameters

Several factors analyzed were found to have significant effects on seedling biometric parameters (Figure 2).

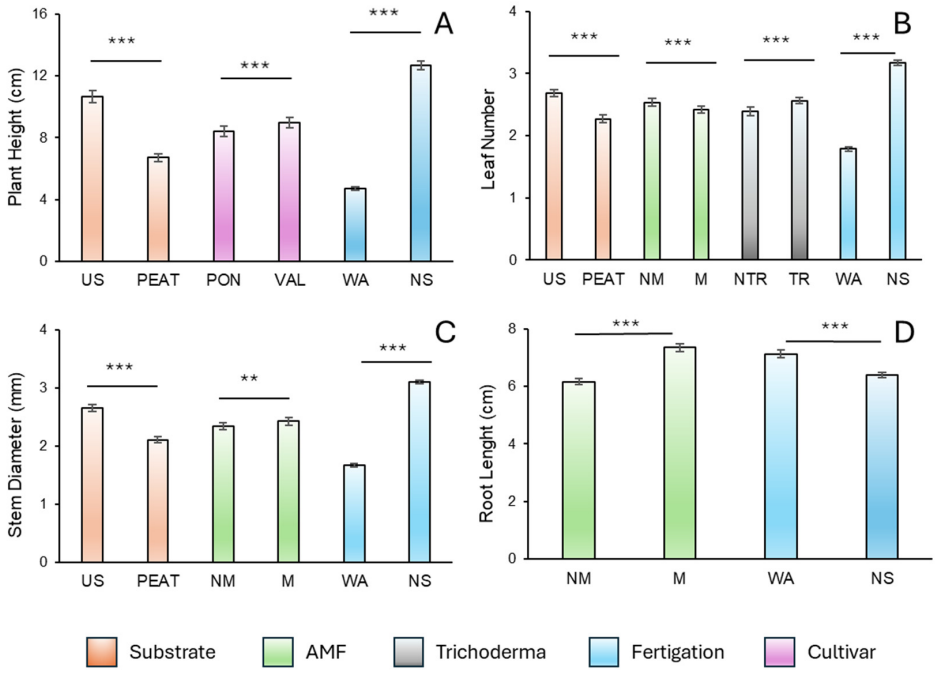


Figure 2. Effect of the five treatment variables on: plant height (**A**), leaf number (**B**), stem diameter (**C**), and root length (**D**). Histograms represent the average values, and error bars represent the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD). **: $p \leq 0.01$. ***: $p \leq 0.001$. US = forest soil, PEAT = peat substrate; NM = no mycorrhization; M = no mycorrhization; NTR = no Trichoderma; TR = with Trichoderma; WA = only water; NS = nutrient solution; PON = Pony; VAL = Valiente.

The factor that played a significant ($p \leq 0.01$) role in all the parameters analyzed was fertigation. Indeed, the use of the nutrient solution at 1200 µS/cm (NS), with respect to plain water (WA), resulted in seedlings with an average height 170% higher, a 77.5% increase in the number of leaves, an 86.2% increase of the stem diameter, but a 10.2% reduction of root length.

The other factor significantly affecting most parameters was the substrate. With the adoption of forest topsoil (US), seedlings showed an increase in height (+58.6%), in number of leaves (+18.3%), as well as in stem diameter (+25.9%), compared to the use of the commercial peat substrate (PEAT).

The two types of fungi tested in this study introduced differences in some of the parameters. *Trichoderma* (TR) significantly increased the number of leaves by 6.95%, whereas the use of AMF (M) decreased this parameter by 4.35%. The M treatment also increased the stem diameter by 3.56% and the root length by 19.1% with respect to the untreated control (NM).

The use of different cultivars showed effects only in seedling height, where seedlings of the open-pollinated variety Valiente (VAL) were 6.61% taller than those of the Pony variety (PONY).

3.2. Biomass Yield and Partitioning

The use of US increased the total dry weight (TDW) of the seedlings by over 100% compared to PEAT, which, on the other hand, resulted in an increase in the shoot/root ratio (Figure 3). Fertigation (NS) led to an increase of TDW by 163% but a reduction of the dry matter (DM) percentage from 14.4% to 9.50%. NS treatment also increased the shoot/root ratio (+119%) and resulted in a higher dry matter allocation of to shoot and less to the root while the opposite was observed with WA (Figure 3). The Pony variety showed a higher dry matter allocation to roots compared to VAL (58.1% vs. 53.1%), which resulted in a lower shoot/root ratio (0.88 vs. 1.04) (Figure 3). The only significant effect of the TR treatment was noted in the percentage of dry matter, which was reduced from 12.71% to 11.23% compared to NTR (Figure 3). Finally, no effect of AMF on these parameters was observed.

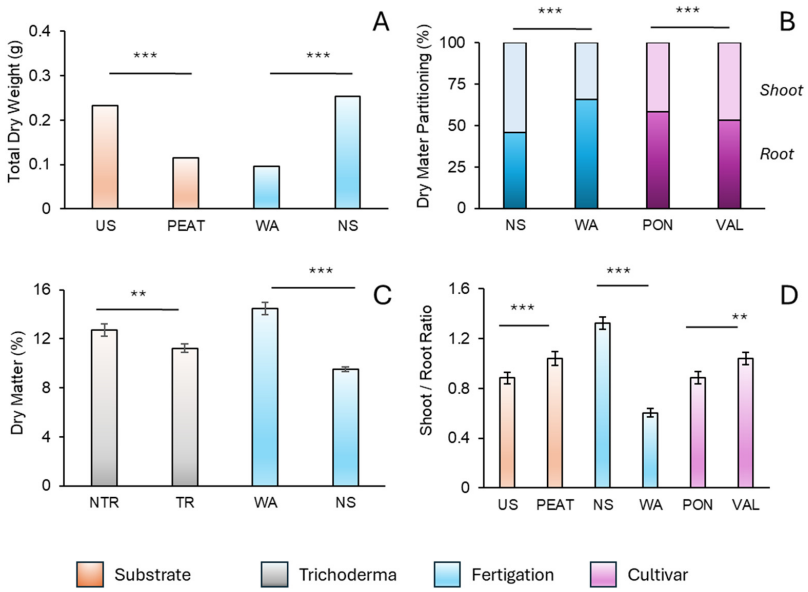


Figure 3. Effect of the five treatment variables on total dry weight (**A**), dry matter partitioning (**B**), percentage of dry matter (**C**), and shoot/root ratio (**D**). Histograms represent the average values, and error bars represent the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD). **: $p \leq 0.01$. ***: $p \leq 0.001$. See the caption of Figure 2 for abbreviations.

3.3. Leaf Temperature Responses

Four out of five variables resulted in significant differences in seedling leaf temperature (Figure 4). The most visible effect was due to fertigation, which was able to decrease the average temperature by 1.98 °C. Forest soil also decreased temperature (−1.03 °C), as did *Trichoderma* application (−0.45 °C). Mycorrhiza application, on the other hand, resulted in seedlings with a higher leaf temperature of 1.17 °C.

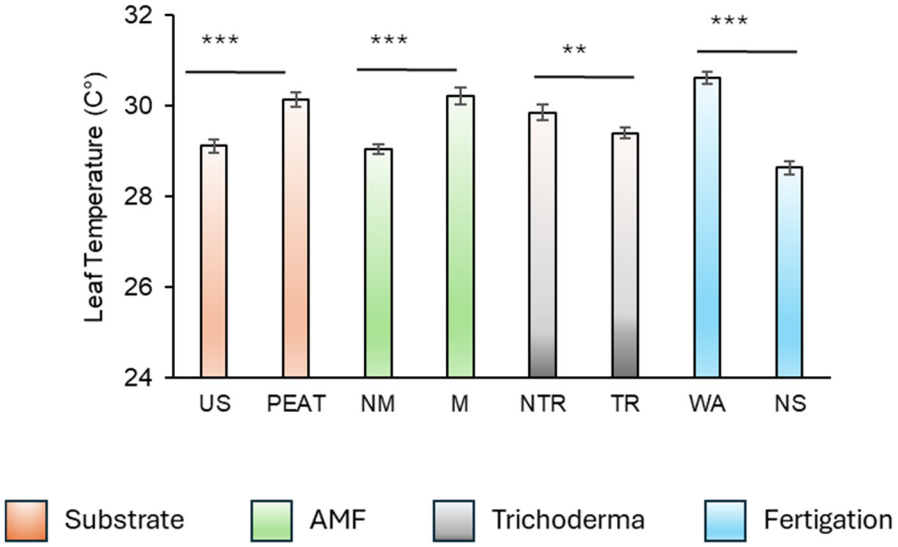


Figure 4. Leaf temperature parameter recorded for the five treatment variables. Histograms represent the average values, and error bars represent the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD). **: $p \leq 0.01$. ***: $p \leq 0.001$. See the caption of Figure 2 for abbreviations.

3.4. Qualitative Parameters

The contributing factors that had the most significant effects on the qualitative parameters were substrate and irrigation (Figure 5). Plants grown in US showed a higher stem diameter ratio, a higher RDW/RL ratio, and the Dickson quality index increased from 0.026 to 0.050 (+90.1%). Regarding the sturdiness quotient, though, it is PEAT that provided the best performance, with a 0.34 value compared to 0.28 of US. The same pattern was observed when fertigation (NS) was adopted instead of water (WA): an increase in the stem diameter ratio (+16.5%), RDW/RL ratio (+107%), and Dickson quality index (+64.5%) with a concurrent decrease in the sturdiness quotient of 29.2%. The VAL cultivar featured a decrease of 5% in the sturdiness quotient, while M application resulted in a reduced RDW/RL ratio by 23.3% (Figure 5).

3.5. Significant Interactions Among Studied Variables

The analysis revealed the multiple interactions among the variables for several of the parameters. The Substrate × Fertigation interaction (Figure 6) showed that fertigation has led to a higher increase of plant height when peat was adopted (173% vs. 168%). The NS fertigation resulted in a more consistent increase of the stem diameter ratio in PEAT than in US (28.6% vs. 6.34%), a lower leaf temperature (−2.51 °C vs. −1.45 °C), and a higher shoot/root ratio (+178% vs. +70%). The irrigation with only water (WA) resulted in the highest dry matter allocation to root and the lowest to shoot, regardless of the adopted substrate. At the opposite, NS with PEAT showed the highest allocation to shoot and the lowest to root (Figure 6).

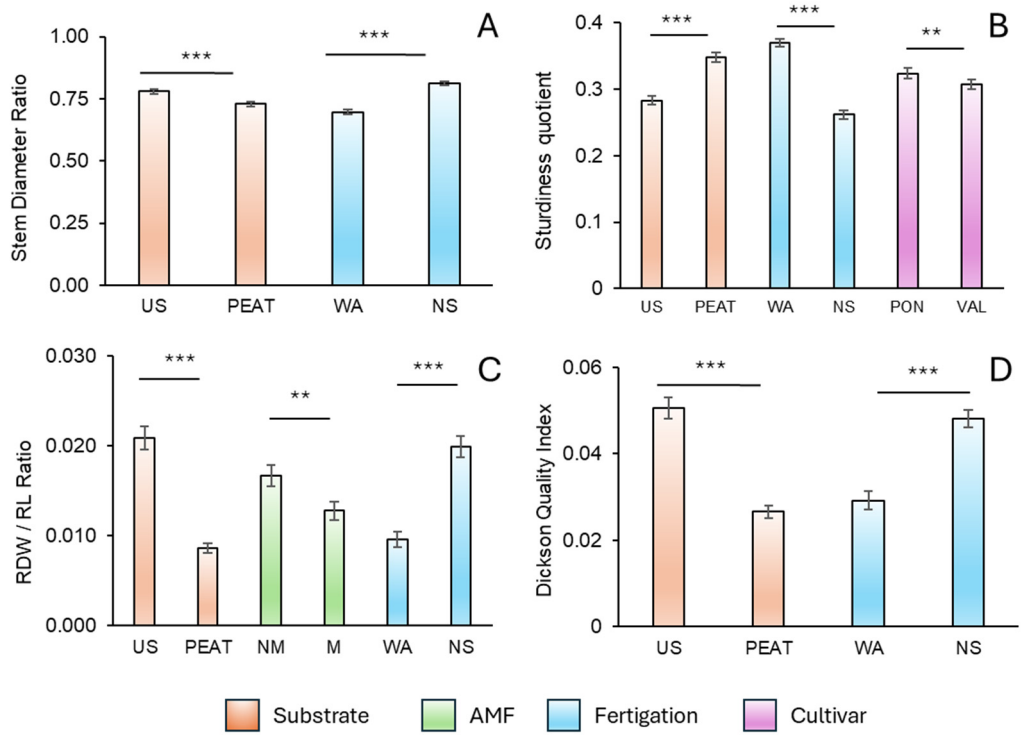


Figure 5. Effect of the five treatment variables on stem diameter ratio (**A**), sturdiness quotient (**B**), root dry weight/root length ratio (**C**), and Dickson Quality Index (**D**). Histograms represent the average values, and error bars represent the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD). **: $p \leq 0.01$. ***: $p \leq 0.001$. See the caption of Figure 2 for abbreviations.

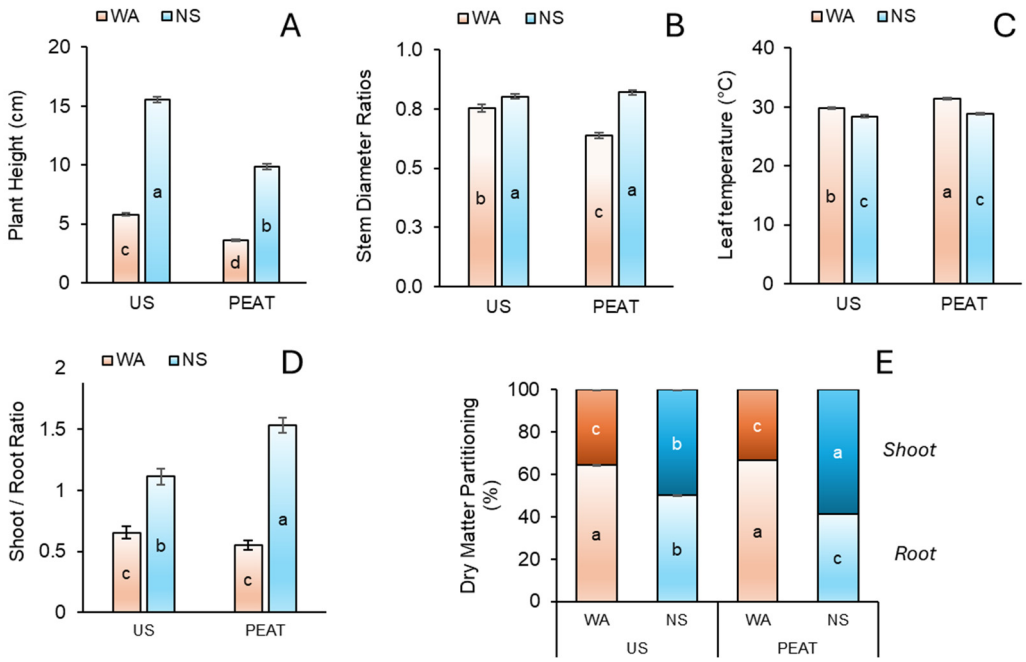


Figure 6. Effect of the substrate × irrigation interaction on plant height (**A**), stem diameter ratios (**B**), leaf temperature (**C**), and shoot/root ratio (**D**), percentage of dry matter partitioning (**E**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

The positive effect of M on seedling height was shown in US but not in PEAT (Figure 7). Differently, only with PEAT substrate, leaf temperature and dry matter differed according to AMF treatment, with M showing the higher temperature and the lower dry matter percentage (Figure 7).

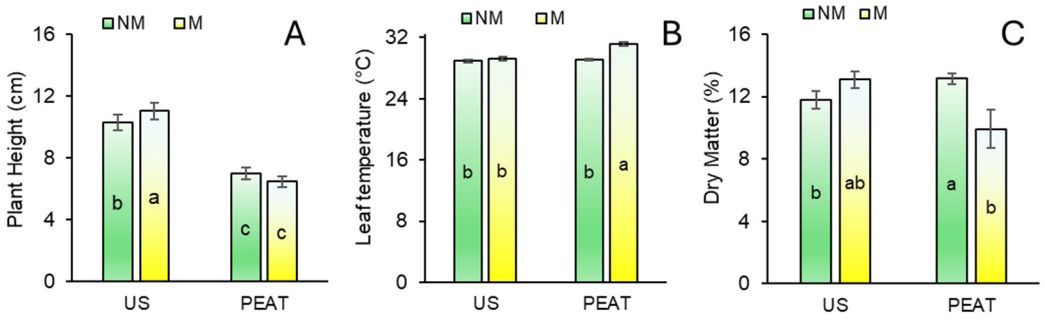


Figure 7. Effect of the substrate × AMF interaction on plant height (**A**), leaf temperature (**B**), and percentage of dry matter (**C**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

A significant interaction between substrate and *Trichoderma* treatment on affecting leaf number, root length, and the RDW/RL ratio was also observed (Figure 8). With PEAT, TR significantly increased leaf number (+14.7%) while reducing root length (−9.46%). Interestingly, with TR, the root dry matter/root length ratio decreased by 23.6% when US was used as a substrate, while it increased by 34.3% using PEAT substrate. The Dickson quality index actually increases with *Trichoderma* application when we use peat (+16.6%) (Figure 8).

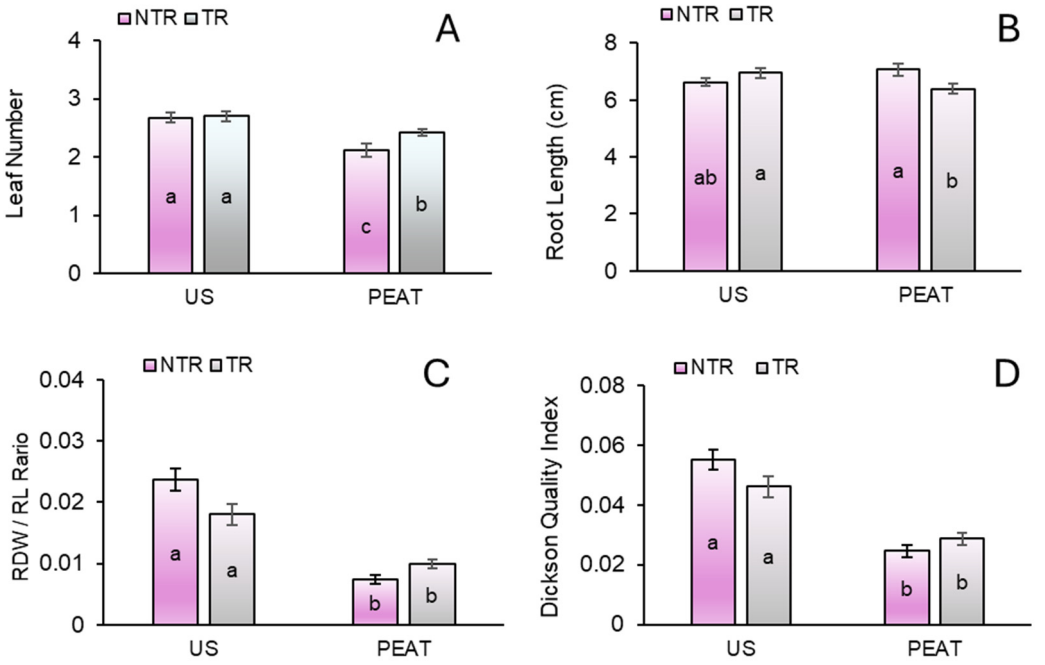


Figure 8. Effect of the substrate × *Trichoderma* interaction on leaf number (**A**), root length (**B**), root dry weight/root length (**C**), and Dickson Quality Index (**D**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

Trichoderma × fertigation interaction significantly affected three morphological plant parameters. For all plant height, leaf number, and stem diameter, NS strongly increased values, especially when *Trichoderma* was not applied (Figure 9).

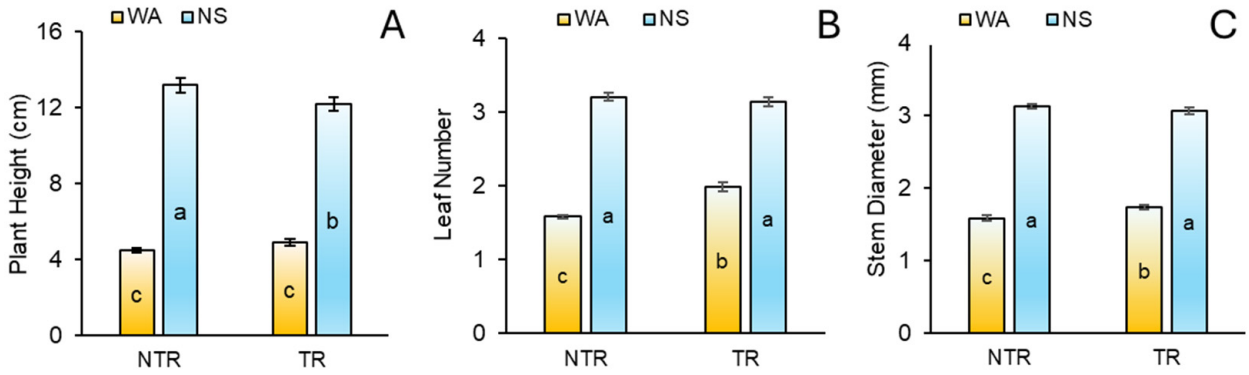


Figure 9. Effect of the *Trichoderma* × fertigation interaction on plant height (**A**), leaf number (**B**) and stem diameter (**C**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

AMF × Fertigation interaction (Figure 10) played a role in root length and leaf temperature. For both parameters, the reduction on values with NS compared to WA is much more evident in AMF presence.

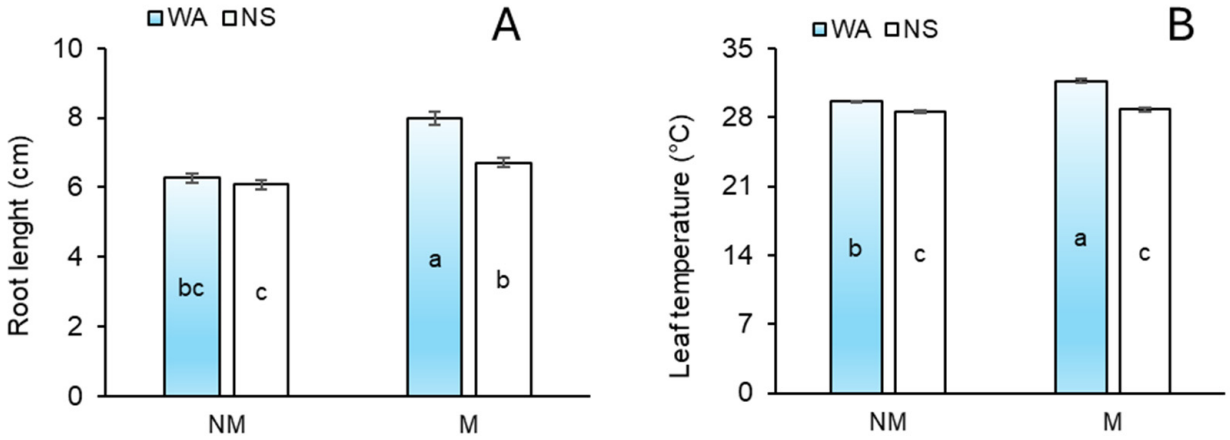


Figure 10. Effect of the AMF × fertigation interaction on root length (**A**), and leaf temperature (**B**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

For the RDW/RL ratio and Dickson quality index, the VAL variety performed worse in US substrate compared to PON while performing better in PEAT. PON and VAL showed similar shoot/root ratios as PEAT substrate was, but lower values of PON compared to VAL in US substrate. This is reflected in the dry matter partition (Figure 11).

Cultivars also showed different responses in dry matter percentage according to AMF treatment. AMF application in PON led to a significant increase in the dry matter content while it was ineffective in the cultivar VAL (Figure 12).

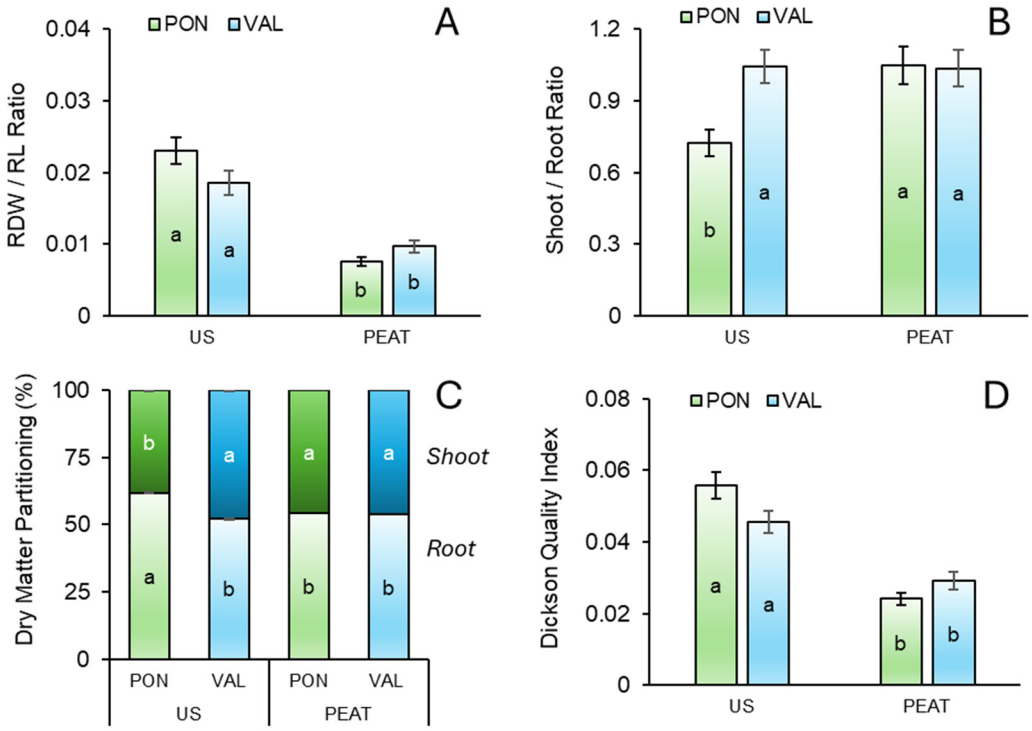


Figure 11. Effect of Substrate × variety interaction on root dry weight / root length ration (**A**), shoot/root ratio (**B**), dry matter partitioning (**C**), and Dickson Quality Index (**D**). Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

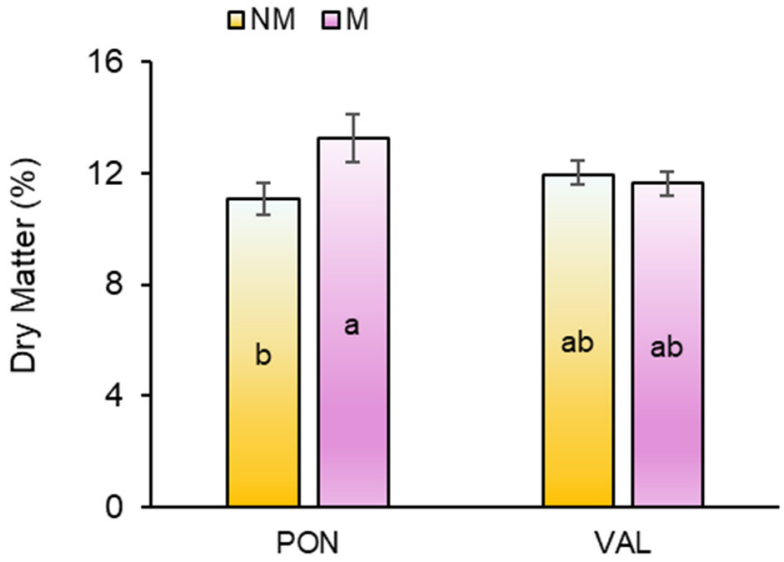


Figure 12. Variety × AMF interaction on percentage of dry matter Histograms represent the average value, and error bars indicate the standard error mean (SEM). Only significant differences for $p \leq 0.01$ are reported, resulting from the Tukey's honestly significant difference test (HSD) for $p \leq 0.01$. Different lowercase letters indicate significant differences. See the caption of Figure 2 for abbreviations.

3.6. Principal Component Analysis (PCA)

In the principal component analysis, two principal components explained 68.4% of the total variance of the data. Separate clusters, with a confidence level of 95%, were

observed for substrate and fertigation treatments, as visible in the Supplementary Materials: Figures S2 and S3. No visible cluster separation could be detected in response to *Trichoderma*, AMF or cultivar treatments. Group separation could also be evidenced when plotting substrate × fertigation treatment subgroups (Figure S4).

4. Discussion

Our results confirm that some of the proposed agronomic practices tested allowed for an increase in the quality of tomato seedlings. In particular, the choice of fertigation and substrate was particularly important for morphological, physiological, and quality plant parameters.

4.1. Fertigation

Tomato seedlings irrigated with nutrient solution displayed more developed aboveground parts in terms of higher plant and stem diameter and stem diameter ratio (a reliable predictor of both field survival and subsequent growth [38], in addition to increasing leaf number and total dry matter. This result is in accordance with previous studies that assessed the effect of different nutrient solutions on tomato seedling quality [39,40]. The reduced percentage of dry matter recorded for seedlings grown under nutrient solution treatment is not necessarily a negative aspect, as it is related to the higher quantity of water retained within the plant tissue, which resulted in higher fresh weight (Figure S5). Similarly to our findings, the percentage of dry matter was found to be lower under higher nitrogen applications, which is a key factor when plants are exposed to heat stress [41]. Accordingly, Luo et al. [42] indicate that nitrogen application is essential for crops to tolerate high temperatures, and seedlings grown without nutrient solution suffered higher stresses. Nitrogen not only increases photosynthetic activity but also allows the plant to neutralize reactive oxygen species, which strengthens leaves' resistance to high-temperature stresses [43]. This results in more turgid stems and leaves, with a higher water content and therefore a reduced percentage of dry matter. Higher leaf water content may also translate into lower leaf temperature, as also experienced in our study, where seedlings grown under fertigation displayed significantly lower leaf temperature (−1.98 °C), conditions that have previously been associated with higher tomato yields [44].

The application of the nutrient solution also affected dry matter partitioning, increasing allocation to the shoot rather than to the roots, as expected from the existing literature [39,45]. This is an adaptative response that, whenever nutrient availability is limiting, drives plants to allocate newer biomass to the organs that are involved in acquiring the resources, i.e., roots [46]. Accordingly, a decrease in root length was also recorded in fertigated seedlings. Another factor that could be involved in this response may also be related to increased salinity forming at the bottom of the trays, a known factor to cause root development reduction [47]. Fertigation, however, achieved a minor sturdiness quotient since the sharp rise in seedling height was not followed by such a significant increase of stem diameter while starving seedlings resulted in stunting plantlets. On the other hand, the overall higher growth (e.g., leaf number) obtained by fertigated plants might have caused reciprocal shading, as well as possible changes in the light composition (e.g., increases in the far red:red ratio within the canopy), which may have caused stem elongation as a result of a shade-avoidance syndrome [48]. That said, the value of the sturdiness quotient that is considered acceptable for successful transplanting is smaller than six; therefore, all plants displayed desirable traits [33]. Ultimately, the use of fertigation increased overall seedling quality (i.e., Dickson quality index) and did not present disadvantages, being a simple and cost-effective practice: using the fertilizer used in the experimentation (*Solufeed*

Inicio, with a price of USD 6.96 per kg) [49], a farmer could grow almost 6000 seedlings with 1 kg of fertilizer.

4.2. Substrates

Among the substrates evaluated, the forest topsoil gave better results compared to peat in terms of morphological, biomass, and physiological seedling traits. This is in accordance with other studies that tested alternative substrates to peat and obtained ameliorations. Substrates solely composed of peat produced lower-quality tomato seedlings compared to those mixed with other materials (e.g., zeolite) [50] or amended with sand and manure [51]. This result also has an important practical application. In a context where small-holder farmers are struggling to make an income from farming [3], the use of topsoil not only can produce higher quality seedlings but is also more economically and environmentally sustainable compared to peat when considering small farms [52,53].

4.3. Arbuscular Mycorrhiza Fungi

AMF had a positive effect on root length, suggesting that their presence is beneficial to the root system. This is in accordance with other studies where AMF-inoculated substrates resulted in tomato seedlings' higher root fresh weight [54]. However, in our study we had a lower RDW/RL ratio using AMF due to the absence of significant improvements in plant biomass. A higher increase in leaf temperature was also observed, and this could be due to the higher ($p \leq 0.05$) dry matter content that mycorrhized plants featured. This poses some questions regarding its causes. While this study aimed to find practical solutions for farmers and therefore adopted a simple inoculation method as labelled on the commercial product, more complex AMF-inoculation methodologies might have resulted in better colonization and higher benefits to the seedlings. In fact, application methodology and the presence of conducive material to establish symbiosis are recognized as possible constraints to successful use of AMF in the field [22]. Gómez-Bellot et al. [55] presented similar results, where the presence of AMF improved only a few parameters, suggesting that the low percentage of colonization can occur and that AMF effectiveness in field conditions is slower and dependent on several and uncontrolled conditions that may emerge along cultivation [55].

4.4. Trichoderma

Application of *Trichoderma* did not result in significant improvements of plant traits related to biomass. However, plantlets treated with *Trichoderma* displayed a higher number of leaves, a lower percentage of dry matter (higher fresh weight) and lower leaf temperatures. Beneficial effects of *Trichoderma* on tomato seedling height, crown diameter, and shoot and root fresh weight were recorded using substrate amendment in standard conditions (+78.5%, +82.3%, +463%, and +210%, respectively, as the average of three *Trichoderma* spp. isolates) [56] and on height, collar diameter, and fresh/dry weight ratios of leaves and roots in drought-stress conditions (about +50%, +70, +35, and +40%, respectively) [57]. The application method may have played a role in our limited growth improvements. While in Cornejo-Rios et al. [57] the application methodology was similar, based on soil amendment, Rasool-Azarmi et al. [56] utilized sterilized wheat grains as a medium, allowing a longer inoculation of 2 weeks and evaluating seedlings 45 days after planting. Favorable conditions and timing are essential factors for the successful establishment of *Trichoderma* spp. [17], and the short duration of the experiment, as well as the absence of the most adequate conditions, might have reduced visible beneficial effects. Considering the major role of *Trichoderma* spp. as biological control agents against plant pathogens [17,19,58], more beneficial effects could have emerged later, after transplanting.

Climatic conditions might have also influenced the growth of both *Trichoderma* and AMF, reducing their expected beneficial effects on seedling growth. There is a positive linear correlation between soil moisture level and *Trichoderma* spp. germination [59], and the growth rate of *Trichoderma asperellum* is reduced in substrates where water is scarce [60]. The climatic conditions experienced in our study, characterized by high temperatures and low RH, could have influenced the soil water availability between irrigation events and therefore compromised the colonization of *Trichoderma* and AMF in the substrate.

4.5. Interactions

The analysis of interactions revealed some interesting findings. For instance, fertigation showed greater benefit if applied with peat. Although the highest absolute values of several parameters were found from NS with forest topsoil, the use of fertigation often resulted in a higher benefit when peat was used as substrate (e.g., plant height, stem diameter ratio, leaf temperature), resulting in a relatively higher growth of above-ground plant organs compared to the root system (i.e., higher shoot/root ratio and dry matter allocation to shoot). This response is likely due to the higher nutrient availability in the US substrate, as results from the much higher EC of this substrate compared to peat. It is therefore crucial to transfer to local farmers, some of whom are already used to cultivating in peat but without fertigating, that the use of the nutrient solution greatly increases their seedling quality.

The application on mycorrhiza showed different interactions. It positively affected plant height only in forest soil, while it negatively impacted dry matter and leaf temperature (i.e., reduced the first and increased the second one) in peat. It is generally accepted that mycorrhization is especially effective in low-fertility soils, as proven, for instance, in coffee and melon [4,61,62]. Mycorrhization also increased root length, particularly when only water, and not NS, was provided, but, conversely, increased leaf temperature. Results are overall in agreement with Johnson et al. [63], which suggests soil fertility to be a key controller of mycorrhizal cost–benefit and colonization, with beneficial effects of mycorrhiza being shown only when there is the presence of the basic nutrients in the soil [63]. However, their study also revealed that a higher availability of a nutrient (i.e., nitrogen) can increase root colonization, and thus the plant biomass, depending upon the shortage of another (i.e., phosphorus) [63]. Hence, it is possible that in our experiment, US soils, although richer in nutrients, resulted overall in more unbalanced. A more significant increase in root length if irrigating with plain water when seedlings were mycorrhized was observed: this is somewhat expected as it is in accordance with the law of the minimum proposed by Johnson et al. [63]. Finally, a better response was also shown in the Pony cultivar in terms of percentage of dry matter but not from the cultivar Valiente. This is no surprise either, as plant responses to treatments are often species- and variety-dependent [64].

Trichoderma seems to have a positive effect on seedling morphological parameters (i.e., plant height, leaf number, and stem diameter) when only water was applied. This is in accordance with previous studies, which demonstrated the *Trichoderma* spp. ability to enhance nutrient uptake and growth of tomato plants [58] and specifically in conditions of low fertilization input [61,65]. However, when *Trichoderma* application is coupled with NS irrigation, these effects are diminished, again since it is in situations of nutrient deficiency and stress that beneficial effects of the fungi are most clearly manifested. In fact, greater results on plant growth have been demonstrated when *Trichoderma* is applied in synergy with biofertilizers [17,66], but the same was not true when applied with the conventional chemical fertilizer.

The positive response to *Trichoderma* in conditions of low fertilization is in accordance with the response to the substrate, as the one with lower nutrient content (peat) resulted in

an increase of leaf number, RDW/RL ratio, and Dickson QI. The different cultivars were a factor we introduced to provide farmers with better advice and possibly promote an open-pollinated cultivar (Valiente), given the economic advantage that lower-cost seeds can offer to small farmers. The results of the experiment showed that the Pony hybrid cultivar had the overall better performance (e.g., higher plant height, higher dry matter allocation to roots). Furthermore, Pony also exhibits better performance in the presence of forest soil, but in the presence of peat (far more common than US among local farmers), it is the Valiente cultivar that shows higher root quality and Dickson QI. However, the parameters affected by the cultivar were not many; thus, for a better indication to the farmer, the post-transplant performances should also be analyzed.

4.6. Dickson Quality Index

The Dickson quality index provides an objective assessment of seedling quality in forestry, floricultural, and horticultural crops [32,67–69] and is an effective tool to predict yields [50,68]. Our study demonstrated that at initial growing stages, AMF and cultivar do not play a relevant role in producing high-quality seedlings, while the adoption of the appropriate substrate and irrigation solution is essential. *Trichoderma* did not play a role as a single factor, but it was noticeable that seedling quality increased when applied in the presence of peat.

Indeed, the adoption of forest topsoil (US) and NS significantly increased DQI, and the treatments with the highest DQI were cultivated in these conditions, regardless of AMF and *Trichoderma* treatments. Additionally, the interaction analysis revealed that the combination of peat and water solution is particularly unrecommended to produce quality seedlings, despite the fact that locally it is a common practice.

A consideration should be made on the seedling quality parameters that we adopted (stem diameter ratio, sturdiness quotient, RDW/RL ratio, and DQI) to have stronger evidence of the treatment's effects. As stated, DQI is one of the more recognized tools for assessing seedling quality, and, in fact, its results were often in accordance with those of the stem diameter ratio and the RDW/RL ratio. Interestingly, the values of the sturdiness quotient were instead in contrast with those of the DQI. It is likely that the quality indexes should be considered carefully when comparing treatments or combinations of treatments that may lead to strong differences in plant growth. Here, for instance, much greater sturdiness values were attributed to seedlings grown in US with NS rather than in peat with WA, but this is because the latter were basically stunted seedlings, which do not necessarily reflect real quality.

5. Conclusions

In this study, the effect of *Trichoderma* and AMF on tomato seedling production under semi-arid conditions of the Trifinio region was investigated and tested along with the main cultural practices.

Overall, the use of symbiotic fungi had no tangible impact on seedling development when applied solely, at least in the initial stage of development. These minor visible effects could be associated with either a low colonization—which unfortunately was not measured along the study—or the short duration of the experiment—which was limited to the nursery stage, while more relevant effects could emerge along the cultivation cycle. Indeed, with reference to *Trichoderma*, it was possible to elaborate that for tomato nurseries it is advisable to apply it in the presence of peat, as it becomes unnecessary and at times even harmful to plant development in the presence of an already rich substrate such as forest soil. On the other hand, whenever access to techniques that increase the availability of nutrients, such as fertigation or forest soil, is not possible, the application of *Trichoderma* does provide benefits

to the plant. When irrigation with plain water occurred, *Trichoderma's* applications increased the height, stem diameter, and number of leaves of the plant. Although the application of AMF increased root length, this did not translate into a real increase in biomass or improvement in plant development, regardless of the agronomic factor with which it was associated. Again, the simplicity of the application method (to meet farmers skills) and the short time given to the fungi to colonize probably influenced the results, which indicated that it was not convenient to apply AMF to tomato seeds in nurseries. The research pointed out how, to produce quality tomato seedlings, the most important agronomic choices (across the hereby tested conditions) were to use a 1200 μS/cm nutrient solution and the forest topsoil as substrate. These two factors alone allowed for the improvement of biometric factors such as plant height and stem diameter, but also increasing the total dry weight and decreasing leaf temperature, hence, improving the seedlings' quality and the probability of successful transplanting. Such findings can support local farmers in their decision-making process, promoting quality tomato seedling production. Further research should validate the results at the seedling stage by correlating them with actual productive performances along the crop cycle. To this end, it is advisable as well to test different methodologies for *Trichoderma* and AMF inoculation, as well as to monitor and validate the actual root colonization rate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15020392/s1, Table S1. Summary of treatments applied in the experiment. Figure S1. Graphical representation of the experimental design. Figure S2. PCA plot showing the weights for principal components of the analysis for the 22 parameters measured in tomato seedlings for substrate treatment classification factor. Figure S3. PCA plot showing the weights for principal components of the analysis for the 22 parameters measured in tomato seedlings for fertigation treatment classification factor. Figure S4. PCA plot showing the weights for principal components of the analysis for the 22 parameters measured in tomato seedlings for the interaction "substrate × fertigation" classification factor. Figure S5. Effect of the substrate and the fertigation treatments on the total fresh weight.

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