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Research Article

Effect of Endophytic *Trichoderma* sp. Strains on the Agronomic Characteristics of Ecotypes of *Theobroma cacao* L. under Nursery Conditions in Peru

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Peru is one of the main producers of fine aroma native cacao, expanding its areas considerably in recent years, which makes it necessary to seek adequate management alternatives to obtain advantageous yields. The present work had the objective of testing the influence of *Trichoderma* sp. endophytic strains on the agronomic characteristics of ecotypes of *Theobroma cacao* L. under nursery conditions, Cajaruro district, Utcubamba, Amazonas, Peru. The Trichoderma strains evaluated were *Trichoderma breve*; *T. harzianum*; *T. longibrachatum*; *T. afrojarzianum,* and *Trichoderma* sp. which were inoculated on cocoa seedlings of ecotypes CCN51; TCHS565; and Nativo fino de aroma. The variables evaluated were chlorophyll indices (at 30, 45, 60, and 70 days after planting), percentage of endophyte colonization, root hair development, trichomes on stems, and agronomic characteristics (plant height, number of leaves, stem diameter, root dry and fresh weight, root size, etc.). The results showed that the application of *Trichoderma breve* in the fine aroma native cocoa ecotype (T12) presented the highest chlorophyll index at 75 days after planting (DAP) with 43.53 ± 1.59 and 49.77 ± 2.42 for the apex and leaf base, respectively, with a percentage of colonization in the root hairs of 66.67%, and with better characteristics for the number of leaves with 12.00 ± 3.46. T12 showed positive influences for plant height, leaf number, and chlorophyll index. Treatments based on *T. harzianum* + CCN51 (T5) and *T. afroharzianum* + TSHS565 (T6) showed 100% colonization of the root hairs and trichomes on stems. Endophytic Trichoderma fungi are an alternative for organic production of fine aroma cocoa in Peru, improving the agronomic characteristics of the crop.

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the most important crops worldwide, with predominance in tropical countries developed in different Agroforestry Systems (AFS) with greater predominance under shade [1]. Latin America has a good geographical position, diversity of ecosystems, and genetic resources, which is why it is positioned as the main supplier of fine aroma cocoa in the world; the productivity of the crop varies in each country: the countries with the highest productivity are Ecuador and Peru achieving levels between 600 kg/ha and 700 kg/ha [2].

Peru exports 75% of fine aroma cocoa, having differentiating attributes against cocoa from Asia and Africa, knowing that 60% of the existing cocoa genetic material in the world is found in Peru among the varieties: Trinitario 53% in Junín; Forastero Amazónico 37.3% in Cusco and Ayacucho; and criollo 9.4% in the northern area of San Martin, Amazonas, and Cajamarca [3]. However, in order to preserve the positioning, constant studies are carried out in

fine aroma cocoa with alternative for agroexport improving quality and productivity [4].

Cocoa plantation soils harbour different microorganisms that help the development of the crop; among them, the Trichoderma fungus, being a group of microorganisms that inhabit the soil naturally, is widely distributed throughout the world; they are usually found in symbiosis with some plants and in the bark of decaying wood [5]. This fungus is used as a broad-spectrum biocontrol agent for the control of different fungal diseases and also as a very important factor in the stimulation of root growth [6].

The mechanism of action of Trichodermas is by entering the interior of the plant tissue (roots, stems, and leaves) and establishing itself in the intercellular spaces without inducing symptoms or visible signs, differentiating them from endomycorrhizae by not forming pelotons or modifications inside the cell, thus characterizing them as a group of nonmycorrhizal endophytic fungi [7, 8].

Plant species such as cocoa host endophytic fungi that can develop in different plant tissues [9]. These fungi contribute to soil fertility, structure, and biodiversity, achieving a symbiotic effect between plants and microorganisms, offering benefits to the host plant such as growth promotion, biocontrol, disease suppression, and stress tolerance, thus contributing to the stability of agroecosystems [10–12].

In addition, there are investigations that evaluated Trichoderma strains, obtaining good results in relation to their antagonistic potential and role as promoters for the improvement of some agronomic characteristics such as the root system, plant height, stem diameter, productivity, and chlorophyll index, among others [13]. In addition, the study of diverse strains is important as some play a key role in plant development and plant responses to pathogens and abiotic stresses, while others produce interesting secondary metabolites [14].

On the other hand, studies such as those of Topolovec-Pintarić [15] have been studying Trichoderma species such as *T. hamatum, T. harzianum, T. polysporum,* and *T. virideare,* which are being widely used in the preparation of biopesticides and biofertilizers, as they are potent enough in the colonization of root tissues; in addition, they interact with the host plant.

Against this background, the present research seeks to test the influence of endophytic *Trichoderma* sp. strains on the agronomic characteristics of ecotypes of cacao under nursery conditions in Peru.

2. Materials and Methods

The trial was conducted in the nursery of the Research Institute for the Sustainable Development of Ceja de Selva (INDES-CES), of the National University Toribio Rodriguez de Mendoza (UNTRM), located in the district of Cajaruro, Utcubamba province at an altitude of 474 m. a. s. l., located at longitude −78°24′49.876 W and latitude −5°45′24.306 S with a monthly rainfall of 27.7 mm [16], with an average temperature from March to June 2021 of 23°C, and a relative humidity of 85%.

The agronomic work in the nursery was done manually and irrigation was done at the time of planting and on a monthly basis because the climatic factors were favorable for not making frequent irrigations.

2.1. Biological Material. The Trichoderma strains were obtained from the collection of antagonistic fungi of the Research Laboratory of Plant Health of the Research Institute for Sustainable Development of Ceja de Selva (INDES-CES), which are conserved at −4°C. The species used for the research work were: *Trichoderma breve* K. Chen and W.Y. Zhuang. (AP1M5-C1); *Trichoderma harzianum* Rifai. (AP2M1-C1); *Trichoderma longibrachatum* Rifai. (F21M5); *Trichoderma afrojarzianum* P. Chaverri, FB Rocha, Degenkolb, and I. Druzhinina (F16M3); and *Trichoderma* sp. (CP24-6).

The cocoa clones were obtained from the germplasm bank of INDES-CES: CCN51; TCHS565; and Nativo fino de aroma located in Naranjos Alto, Cajaruro, Utcubamba, Amazonas.

2.2. Activation and Propagation of Trichoderma. The sterile rice-based substrate was prepared. For this, 300 g of rice were weighed and placed in 11×16-inch polypropylene bags, previously soaked for 20 minutes in boiled water; then the bags were stapled at the top edge and sterilized in an autoclave at 121°C, 15 pounds of pressure for 45 minutes. Subsequently, the bags were removed and the rice was allowed to cool, which was moistened with sterile distilled water to be inoculated with the study strains [17].

The activation of Trichoderma strains was carried out in plates containing Papa Dextrose Agar (PDA), for a period of 15 days at a temperature of 25°C with 12-hour photoperiods (fluorescent white light/darkness) [18]. Subsequently, 5 discs of 0.5 mm in diameter were removed from the Petri dish and placed in bags with sterile rice substrate. Ten ml of distilled water was added to moisten all the substrate and homogenized; this process was carried out inside a laminar flow cabinet. Finally, the bags were closed and incubated for 15 days at a temperature of 27°C. Spore counting was performed before taking to the field, based on serial dilutions, and when this showed a concentration of 107 spores/g, they were taken out to be transferred to the nursery [19, 20].

The nursery substrate consisted of a mixture of 100 kg of agricultural soil, 50 kg of river sand, and 50 kg of peat (2 : 1 : 1 ratio). The agricultural soil was collected from the area where the nursery was located (Utcubamba); the predominant soil types are Calera I and Pirias—Pericos Rojos [21, 22]. This mixture was analyzed at the Soil and Water Research Laboratory of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas. It presented physicochemical characteristics such as pH of 6.5, electrical conductivity of 0.30 dS/m, 4.6% organic matter, 0.23% nitrogen, 10 ppm phosphorus, 180 ppm potassium, and a sandy loam texture. This substrate was previously sterilized in a vertical autoclave at 121°C, 15 pounds of pressure for 30 minutes. Subsequently, it was placed in 6x 9-inch polyethylene bags.

Cocoa cobs from the INDES-CES germplasm bank were selected for seed extraction, which were chosen from the middle third of the cob. The seeds were submerged in sterile distilled water for five days until the appearance of the embryo; this procedure was carried out in order to accelerate germination. Subsequently, the seeds were immersed for 10 minutes in 2% sodium hypochlorite, washed three times with sterile distilled water, and placed in the substrate.

One hundred milliliters of the bioconcentrate was prepared per bag (70 grams of Trichoderma plus 30 ml of agricultural oil to facilitate the dispersion of spores) [23]. This bioconcentrate was applied directly at the time of planting; the frequency of application was every 15 days for 75 days according to the methodology of Santander & Olave [24].

2.3. Experimental Design. A Completely Randomized Design (CRD) with 5×3 factorial arrangement (5 strains of Trichoderma: *T. breve*, *T. harzianum*, *T. longibrachatum, T. afrojarzianum,* and *Trichoderma* sp. and 3 ecotypes of cocoa: CCN51; TCHS565; and Nativo Fino de Aroma) was used, making a total of 15 treatments (Table 1), with three replications, having a total of 45 experimental units consisting of 10 plants each.

2.4. Parameters Evaluated

2.4.1. Chlorophyll Index. This parameter began to be determined from 30 days after sowing (DDS) with a frequency of every 15 days until completing 75 DDS. For this, a measurement was taken at the apex and basal part of the fourth leaf from above for each treatment; this was done with a chlorophyll meter model SPAD PLUS 502 [25].

2.4.2. Percentage of Endophytic Colonization and Development of Root Hairs and Trichomes in the Stem. An evaluation was performed 75 days after seedling emergence, assessing endophytic colonization and development of root hairs from trichomes on the stem adapting the methodology of Prasanna et al. [26]. Stem and root pieces (1 cm) were disinfected with 5% sodium hypochlorite for three minutes, followed by immersion in 75% alcohol for one minute, and then rinsed in sterile distilled water and dried with sterile paper towel. The inoculation of the root and stem fragments was done in Petri dishes with water agar content for 16 hours and incubated in the dark at $26 \pm 0.2^{\circ}$C. Subsequently, fungi emerging from the root hairs and trichomes were collected with the help of forceps and a very fine laboratory brush and inoculated in Petri dishes with PDA and incubated at $27 \pm 0.2^{\circ}$C.

Observations of Trichoderma colonization over the next 15 days on both root and stem were distinguished based on the morphological characteristics presented by the fungus on PDA culture medium [27]. The number of plates that tested positive for Trichoderma development were counted among the total number of replicates (3) worked per hundred.

Table 1: Description of treatments.

Treatments	Trichoderma + Ecotypes
T1	*T. afroharzianum* + CCN51
T2	*T. breve* + CCN51
T3	*Trichoderma* sp. + CCN51
T4	*T. longibrachatum* + CCN51
T5	*T. harzianum* + CCN51
T6	*T. afroharzianum* + TSHS565
T7	*T. breve* + TSHS565
T8	*Trichoderma* sp. + TSHS565
T9	*T. longibrachatum* + TSHS565
T10	*T. harzianum* + TSHS565
T11	*T. afroharzianum* + Nativo fino de aroma
T12	*T. breve* + Nativo fino de aroma
T13	*Trichoderma* sp. + Nativo fino de aroma
T14	*T. longibrachatum* + Nativo fino de aroma
T15	*T. harzianum* + Nativo fino de aroma

2.4.3. Agronomic Characteristics. In the agronomic analysis, plant height was evaluated by measuring from the base of the neck of the plant to the terminal bud with a graduated ruler; the number of leaves was determined by direct counting of leaves present per plant, the stem diameter was considered the basal diameter (5 cm from the base of the stem), and was measured with a vernier. For the determination of root fresh and dry weight, the remaining plants of the research were used (3 replicates per treatment), using the gravimetric method which consisted of drying in an oven at 65°C until a constant weight was reached [28].

2.5. Data Analysis. The assumptions of normality and homogeneity of the data were verified to perform a two-way analysis of variance and Fisher's least significant difference (LSD) mean comparison tests at 0.05% confidence, followed by a principal component analysis and its correlation to evaluate the main significant correlations between the agronomic variables evaluated. Data were analyzed with the help of the statistical software InfoStat/Professional version 2018p.

3. Results and Discussion

3.1. Chlorophyll Index. Chlorophyll indices for the leaf apex and leaf base showed highly significant statistical differences for treatments and days after planting ($p < 0.0001$). Treatment 12 showed the best chlorophyll index results for both leaf apex and leaf base (Table 2; Figure 1(a)).

On the other hand, it is evident that the chlorophyll index has increased as the days of evaluation passed after planting (Figure 1(a)). Similar behavior can be observed with respect to the chlorophyll index at the base of the leaf (Figure 1(b)). Ribeiro et al. [25] mention that the chlorophyll index begins to increase at the beginning of vegetative development due to the start of the photosynthetic process.

The variability in SPAD indices existing among the different treatments is due to the interaction between Trichoderma strains with cocoa ecotypes stimulating growth and vegetative maturation through multiple actions such as

Table 2: Chlorophyll index (SPAD) in the leaf apex and base by treatments.

N	Treatments	Apex $\overline{X} \pm SD$	Base $\overline{X} \pm SD$
T1	*T. afroharzianum* + CCN51	28.4 ± 6.77 def	31.62 ± 8.15 ef
T2	*T. breve* + CCN51	27.58 ± 4.62 f	30.26 ± 5.83 f
T3	*Trichoderma* sp. + CCN51	30.43 ± 8.42 cdef	32.02 ± 7.11 ef
T4	*T. longibrachatum* + CCN51	31.96 ± 7.10 bc	33.21 ± 7.51 cdef
T5	*T. harzianum* + CCN51	33.68 ± 7.82 ab	35.31 ± 7.47 bcd
T6	*T. afroharzianum* + TSHS565	28.16 ± 5.53 ef	31.07 ± 5.90 ef
T7	*T. breve* + TSHS565	30.87 ± 6.14 bcde	33.88 ± 6.85 bcde
T8	*Trichoderma* sp. + TSHS565	29.79 ± 8.09 cdef	31.84 ± 8.26 ef
T9	*T. longibrachatum* + TSHS565	31.77 ± 6.94 bc	34.29 ± 8.20 bcde
T10	*T. harzianum* + TSHS565	32.5 ± 6.29 bc	36.2 ± 7.46 bc
T11	*T. afroharzianum* + Nativo fino de aroma	33.47 ± 7.18 ab	36.72 ± 8.36 b
T12	*T. breve* + Nativo fino de aroma	35.93 ± 8.64 a	40.19 ± 10.59 a
T13	*Trichoderma* sp. + Nativo fino de aroma	31.28 ± 4.31 bcd	33.87 ± 4.86 bcde
T14	*T. longibrachatum* + Nativo fino de aroma	30.03 ± 6.55 cdef	32.29 ± 5.56 def
T15	*T. harzianum* + Nativo fino de aroma	30.33 ± 4.46 cdef	31.85 ± 5.07 ef

Different letters in a column indicate significant statistical differences, $\overline{X}$: mean, SD: Standard Deviation. The probability level is 95% confidence.

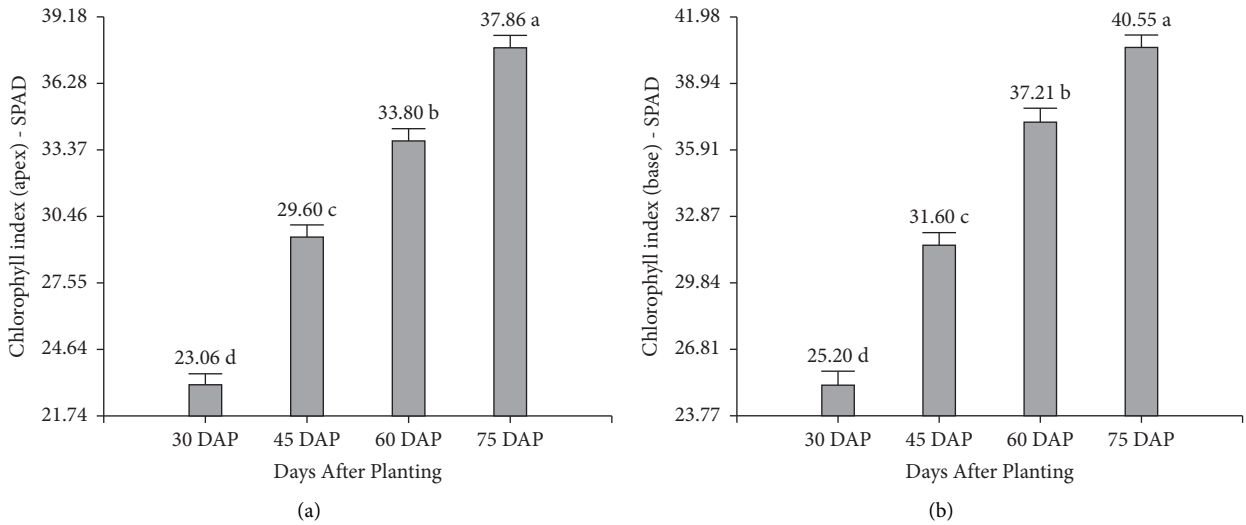

Figure 1: Chlorophyll index (SPAD) for the leaf apex (a) and the leaf base (b) by Days After Planting (DAP). Whiskers show standard deviation and horizontal letters show significant statistical differences. The probability level is 95% confidence.

mycoparasitism, antibiosis, toxin degradation, increased nutrient uptake, solubilization, increased root development, nutrient sequestration, and increased photosynthesis (Ymineni et al., 2019).

There are directly proportional relationships between the chlorophyll indices for the leaf apex and base according to DAP. These data could be attributed to the relationship between photosynthetic efficiency and plant maturity in relation to leaves [29]. It can also be corroborated that the chlorophyll index increases progressively according to the physiological state of the plants [30]. In addition, there may be variability of the chlorophyll index, reporting lower values in dry seasons and higher values in rainy seasons [31]; this variability is explained due to the high plasticity presented by the different cocoa clones [32].

Tezara et al. [33] mention that climatic conditions act favorably or unfavorably on the different clones and cause them to react differently according to the climatic conditions. However, for annual plants such as *Lisianthus,* the values at the beginning of vegetative development present high chlorophyll ranges because they are at their maximum fullness of photosynthetic development; after 63 to 105 DAP, the nitrogen content in the leaves decreases, since this is transferred to the other organs of the plant mainly for the formation of flower buds [25]. Santoyo et al. [34] mentioned that endophytes help plants directly in the uptake of nutrients such as phosphorus, iron, and nitrogen by producing phytohormones such as auxins, IAA, gibberellic acid, and ethylene.

3.2. Percentage of Endophytic Colonization and Development of Root Hairs and Trichomes in the Stem. In response to the percentage of endophytic Trichoderma colonization in the

Table 3: Extent of Trichoderma colonization in the roots and stems of cocoa.

N	Treatments	Colonization (%)	
		Root	Stem
1	*T. afroharzianum* + CCN51	33.33	33.33
2	*T. breve* + CCN51	100.00	33.33
3	*Trichoderma* sp. + CCN51	33.33	33.33
4	*T. longibrachatum* + CCN51	100.00	0.00
5	*T. harzianum* + CCN51	100.00	100.00
6	*T. afroharzianum* + TSHS565	100.00	100.00
7	*T. breve* + TSHS565	33.33	0.00
8	*Trichoderma* sp. + TSHS565	100.00	33.33
9	*T. longibrachatum* + TSHS565	0.00	0.00
10	*T. harzianum* + TSHS565	66.67	100.00
11	*T. afroharzianum* + Nativo fino de aroma	33.33	33.33
12	*T. breve* + Nativo fino de aroma	66.67	0.00
13	*Trichoderma* sp. + Nativo fino de aroma	66.67	66.67
14	*T. longibrachatum* + Nativo fino de aroma	66.67	0.00
15	*T. harzianum* + Nativo fino de aroma	66.67	66.67

Table 4: Mean values and standard deviation of agronomic variables according to the treatments evaluated.

Treatments	Number of leaves (per plant) ? $p = 0.27$ $F = 1.29$	Stem diameter (mm) $p = 0.02$ $F = 2.41$	Plant height (cm) $p = 0.01$ $F = 2.49$	Root size (cm) $p = 0.65$ $F = 0.81$	Fresh weight of roots (g) $p = 0.30$ $F = 1.24$	Dry weight of roots (g) $p = 0.0014$ $F = 3.66$
1	9.33 ± 1.53 a–d	4.04 ± 0.65 cde	22.83 ± 3.01 cde	27.63 ± 5.73 ab	0.87 ± 0.21 b	0.30 ± 0.00 d
2	7.67 ± 0.58 d	3.87 ± 0.85 de	24.40 ± 3.68 b–e	34.77 ± 7.89 a	1.60 ± 0.46 b	0.47 ± 0.15 cd
3	10.67 ± 1.15 abc	4.33 ± 0.22 b–e	27.70 ± 2.25 abc	26.60 ± 0.79 ab	1.63 ± 0.15 b	0.47 ± 0.06 cd
4	10.33 ± 1.53 a–d	4.64 ± 0.62 b–e	27.57 ± 2.80 abc	28.00 ± 6.42 ab	1.73 ± 0.58 b	0.43 ± 0.12 cd
5	9.67 ± 1.53 a–d	4.40 ± 0.28 b–e	29.83 ± 0.50 a	29.20 ± 6.84 ab	1.57 ± 0.21 b	0.60 ± 0.10 bc
6	10.33 ± 1.15 a–d	4.93 ± 0.59 abc	25.87 ± 1.66 a–d	28.60 ± 4.42 ab	1.67 ± 0.12 b	0.50 ± 0.10 cd
7	8.00 ± 1.00 cd	4.86 ± 0.52 a–d	20.67 ± 1.15 e	26.97 ± 0.61 ab	1.47 ± 0.15 b	0.43 ± 0.12 cd
8	9.67 ± 0.58 a–d	4.43 ± 0.12 b–e	26.20 ± 2.61 a–d	27.67 ± 3.75 ab	1.43 ± 0.15 b	0.40 ± 0.10 cd
9	9.33 ± 0.58 a–d	4.94 ± 1.01 abc	28.53 ± 2.82 ab	25.20 ± 1.82 b	2.10 ± 1.40 b	0.97 ± 0.21 a
10	10.00 ± 1.00 a–d	3.77 ± 0.33 e	27.73 ± 2.18 abc	32.67 ± 7.22 ab	1.00 ± 0.17 b	0.27 ± 0.06 d
11	9.33 ± 1.53 a–d	5.19 ± 0.98 ab	21.53 ± 5.00 de	31.63 ± 7.50 ab	2.17 ± 0.80 b	0.43 ± 0.15 cd
12	12.00 ± 3.46 a	4.84 ± 0.23 bcd	27.00 ± 3.55 abc	24.70 ± 4.06 b	1.40 ± 0.53 b	0.47 ± 0.15 cd
13	11.00 ± 2.65 ab	5.85 ± 0.49 a	24.80 ± 2.52 b–e	28.70 ± 6.22 ab	2.47 ± 0.57 a	0.83 ± 0.15 ab
14	9.00 ± 2.65 bcd	4.41 ± 0.80 b–e	23.33 ± 2.08 cde	31.30 ± 5.92 ab	1.87 ± 1.42 b	0.53 ± 0.42 cd
15	8.33 ± 2.31 bcd	4.25 ± 0.43 b–e	23.60 ± 4.80 cde	29.13 ± 2.01 ab	1.30 ± 0.70 b	0.40 ± 0.20 cd

Where: 1 = *T. afroharzianum* + CCN51, 2 = *T. breve* + CCN51, 3 = *Trichoderma sp.* + CCN51,4 = *T. longibrachatum* + CCN51, 5 = *T. harzianum* + CCN51, 6 = *T. afroharzianum* + TSHS565, 7 = *T. breve* + TSHS565, 8 = *Trichoderma sp.* + TSHS565, 9 = *T. longibrachatum* + TSHS565, 10 = *T. harzianum* + TSHS565, 11 = *T. afroharzianum* + Nativo fino de aroma, 12 = *T. breve* + Nativo fino de aroma, 13 = *Trichoderma sp.* + Nativo fino de aroma, 14 = *T. longibrachatum* + Nativo fino de aroma, and 15 = *T. harzianum* + Nativo fino de aroma.

root hairs and stem trichomes, treatments 2, 4, 5, 6, and 8 presented 100% endophytic colonization, and for stem evaluations, the best treatments with maximum colonization were treatments 5, 6, and 10 (Table 3), facilitating the mobilization of Trichoderma throughout the plant as mentioned in the studies carried out by Rosmana et al. [27],

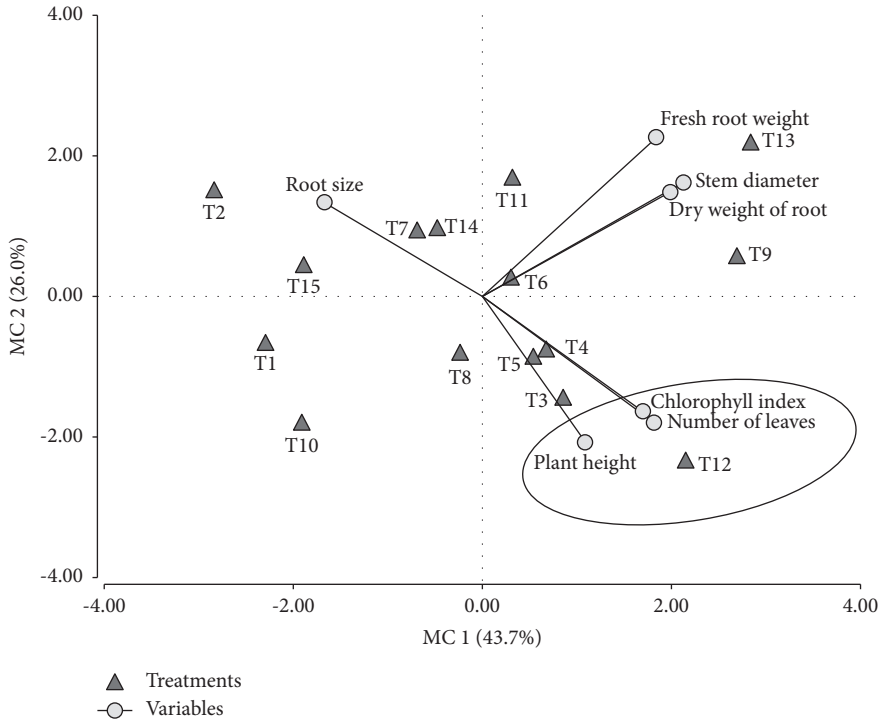

Figure 2: Principal component analysis for cocoa agronomic variables.

who when applying *Trichoderma asperellum* on the leaves of cocoa seedlings verified that Trichoderma is able to spread quickly from the place of its inoculation toward the leaves, stems, and roots of the seedlings, obtaining a colonization of 4% in leaves, 84% in stems, and 32% in roots evaluated in four weeks after the inoculation.

On the other hand, Bailey et al. [35] mentioned that when applying Trichoderma strains and carrying out evaluations of the endophytic association through trichomes, a development of up to 100% of the same strains applied in the plant of origin is obtained. Similarly, Lizarazo et al. [36] when performing the analysis of colonization of different fungi in plants such as *Cattleya trianai* and *Cattleya percivaliana* found that *Cattleya trianai* presented a frequency of colonization in the root tissues of 12.5 and *Cattleya percivaliana* obtained a frequency of colonization in the leaf tissues of 4.2.

3.3. Agronomic Characteristics. In the seedlings evaluated at 90 days, in terms of number of leaves, the best was T12; for stem diameter, the best treatment was T13 (Table 4). The results found for this variable are notably superior to those reported by Chávez et al. [37] who had average results for stem diameter at 120 days of 4.69 ± 0.5 mm in cocoa crosses EEET-558 x CCN51 in Manabí, Ecuador. The differences found could be due to the varieties of cocoa that interact with the different strains of Trichoderma applied because these solubilize phosphate, fix atmospheric nitrogen, and promote the production of iron for the correct growth of the crop [38].

In the present work, in the fine aroma native cacao ecotype, superior results were found to those reported by Meza et al. [22] who when applying different doses of fertilizers obtained seedlings with a stem diameter of 5.63 mm at 74 days of evaluation. This could be due to the fact that Trichoderma has different auxin production mechanisms that when entering in symbiosis with the root improve the agronomic characteristics of the cocoa plant, in such a way that when developing a greater amount of root this has greater ease of absorption of the nutrients available in the soil; in addition, Trichoderma together with the microorganisms creates associations that help increase the rhizosphere of the soil, degrading the organic matter in less time and allowing the plants to extract the nutrients with a greater degree of assimilation [39].

In the evaluations of plant height, T5 recorded the highest value; for the total root size, the superior value was T2. In the evaluation of root fresh weight, T13 presented the highest average with respect to the other treatments, and for root dry weight, T9 showed superiority (Table 4). Camargo and Avila [40] conducted a study in peas with the use of three types of Trichoderma, finding similar behavior with respect to agronomic variables such as plant height, root size, and stem diameter.

Different letters in a column indicate significant statistical differences; the probability level is 95% confidence.

3.4. Principal Component Analysis of Agronomic Variables. The Principal Component Analysis (PC), with its first axis (PC 1) was able to explain 43.5% of the data variability, and the second principal component (PC 2) explained 25.6% of the variability (Figure 2), adding up to a total of 70.9% of explanation of the agronomic variables studied.

Within the analysis of CP, it was found that there was a positive influence of treatment 12 on the variables: number of leaves, chlorophyll index, and plant height; this is due to the fact that Trichoderma stimulates plant growth in addition to the fact that they produce bioactive metabolites that the plant uses as a nutrient [41]. On the other hand, these results coincide with the results reported by Meza-Calderón et al. [42] who found a positive influence between these variables for the evaluation of cacao rootstocks. This could be due to the good development of the plant due to the application of Trichoderma [28].

4. Conclusion

The application of Trichodermas in cocoa ecotypes demonstrated the effect on its different vegetative tissues, showing good agronomic characteristics. The treatment based on *Trichoderma breve* with the fine aroma native cocoa ecotype showed the best results in terms of chlorophyll index and number of leaves per plant; the treatments that presented 100% colonization in the root hairs as well as the trichomes in the stem were *Trichoderma harzianum* + CCN51 and *Trichoderma afroharzianum* + TSHS565. These trials could be complemented by applying these fungi to more cocoa clones at the final field level.

Data Availability

The data used to support the findings of this study are available and can be requested from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest with respect to the publication of this article.

Authors' Contributions

A. C. J. and S. L. conceptualized the study; A. C. J. and M.A. designed the methodology; formal analysis was performed by L.G.B. and C.N.V.; A. C. J., M. A., and M. O. C. were responsible for the research; the original draft was prepared by A. C. J., L. G. B., and M. O. C.; A. C. J., S. L., L.G.B., C.N.V., and M. O. C wrote, revised, and edited the manuscript. All authors have read and accepted the published version of the manuscript.

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