Tropical and Subtropical Agroecosystems

Effect of *Trichoderma*-based microbial bioinputs on growth and seedling quality of banana under nursery conditions †

[Efecto de bioinsumos microbianos a base de *Trichoderma* sobre el crecimiento y la calidad de plántulas de banano en vivero]

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SUMMARY

Background: The nursery stage is critical for producing vigorous banana seedlings before field establishment. However, micropropagated seedlings may show limited early growth during ex vitro acclimatization, making the use of microbial biostimulants a relevant strategy to improve seedling quality. **Objective:** This study aimed to evaluate the effect of *Trichoderma* spp.-based bioproducts on the growth, biomass accumulation, physiological status, and quality indices of banana seedlings under nursery conditions. **Methodology:** Five treatments were evaluated: BIO-TRICH, Trichoplus, Tricomix, YaraMila™ INTEGRADOR™, and an untreated control. At 70 days after transplanting, morphological traits, fresh and dry biomass by organ, SPAD chlorophyll index, tissue water content, root traits, and derived quality indices were determined. **Results:** The *Trichoderma* spp.-based treatments significantly increased absolute growth rate, plant height, pseudostem diameter, total leaf area, total fresh biomass, total dry biomass, and SPAD index compared with the untreated control, with increases of 172.9%–186.5%, 99.9%–107.7%, 65.0%–68.6%, 448.8%–481.8%, 206.6%–256.8%, 166.1%–235.0%, and 45.0%–51.0%, respectively. Tricomix showed the most consistent response, with the highest total dry biomass and Dickson quality index. Tissue water content, total root length, specific root length, and specific leaf area did not differ significantly among treatments. **Implications:** These findings suggest that *Trichoderma* spp.-based bioproducts can strengthen nursery management by improving morphophysiological seedling quality; however, field validation is required to confirm whether these nursery improvements translate into better establishment and productivity. **Conclusions:** *Trichoderma* spp.-based bioproducts, particularly Tricomix, improved the morphophysiological quality of banana seedlings under nursery conditions.
Key words: Dickson quality index; dry biomass; *ex vitro* acclimatization; SPAD index.

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RESUMEN

Antecedentes: La fase de vivero es crítica para producir plántulas de banano vigorosas antes del establecimiento en campo. Sin embargo, las plántulas micropropagadas pueden presentar crecimiento inicial limitado durante la aclimatación ex vitro, por lo que el uso de bioestimulantes microbianos representa una estrategia relevante para mejorar su calidad. **Objetivo:** Evaluar el efecto de bioproductos a base de *Trichoderma* spp. sobre el crecimiento, acumulación de biomasa, estado fisiológico e índices de calidad de plántulas de banano bajo condiciones de vivero. **Metodología:** Se evaluaron cinco tratamientos: BIO-TRICH, Trichoplus, Tricomix, YaraMila™ INTEGRADOR™ y un control absoluto. A los 70 días después del trasplante se determinaron variables morfológicas, biomasa fresca y seca por órgano, índice de clorofila SPAD, contenido hídrico, variables radicales e índices derivados de calidad. **Resultados:** Los tratamientos con *Trichoderma* spp. incrementaron significativamente la tasa absoluta de crecimiento, altura de planta, diámetro del pseudotallo, área foliar total, biomasa fresca total, biomasa seca total e índice SPAD respecto al control absoluto, con aumentos de 172,9%–186,5%, 99,9%–107,7%, 65,0%–68,6%, 448,8%–481,8%, 206,6%–256,8%, 166,1%–235,0% y 45,0%–51,0%, respectivamente. Tricomix presentó la respuesta más consistente, con mayor biomasa seca total e índice de calidad de Dickson. El contenido hídrico, la longitud radicular total, la longitud radicular específica y el área foliar específica no difirieron significativamente entre tratamientos. **Implicaciones:** Estos resultados sugieren que los bioproductos a base de *Trichoderma* spp. pueden fortalecer el manejo de vivero al mejorar la calidad morfofisiológica de las plántulas; sin embargo, se requieren evaluaciones en campo para confirmar su efecto sobre establecimiento y productividad. **Conclusiones:** Los bioproductos a base de *Trichoderma* spp., especialmente Tricomix, mejoraron la calidad morfofisiológica de plántulas de banano bajo condiciones de vivero.

Palabras clave: aclimatación *ex vitro*; biomasa seca; índice de calidad de Dickson; índice SPAD.

INTRODUCTION

Banana (*Musa paradisiaca* L.) is one of the most important fruit crops in tropical and subtropical regions because of its contribution to food security and its economic relevance in national and international markets. Its importance in global trade positions it as a strategic agricultural export commodity (FAO, 2025). In Latin America and the Caribbean, banana is one of the most important fruit crops for production and export. During 2016–2018, the region produced approximately 30 million tonnes of bananas per year and exported an annual average of 13 million tonnes, representing about 80% of global banana shipments and generating approximately USD 5.6 billion per year in export revenue (Altendorf, 2019, Zhiminaicela Cabrera *et al*., 2020). In Ecuador, banana production is a key component of the agri-food and export sectors; in 2024, it reached a harvested area of 175,818 ha and an annual production of 7.5 million tons, while the Musaceae sector contributed USD 3,838.7 million and accounted for 15.4% of the country's non-oil exports (INEC, 2025).

In Ecuadorian banana plantations, field renewal is a fundamental practice; therefore, the use of clonal material obtained through *in vitro* micropropagation is preferred, as it is widely used in modern commercial banana production systems oriented toward export markets (Galan *et al*., 2018). This system enables the production of uniform plant material with high phytosanitary quality by facilitating the multiplication of disease-free banana seedlings, thereby supporting the establishment of new production areas (Darge *et al*., 2026, Shukla *et al*., 2025).

However, during micropropagation and subsequent *ex vitro* acclimatization, the elimination of microorganisms associated with plant material may reduce seedling interactions with beneficial endophytes, compromising biological protection and physiological adaptation during early establishment (Koffi and Declerck, 2015, Xiang *et al*., 2023). Consequently, the nursery stage is a critical phase, as the transition of micropropagated seedlings from *in vitro* conditions to the *ex vitro* environment requires gradual hardening to promote root and leaf development, as well as survival during early establishment (Galan *et al*., 2018, Grzelak *et al*., 2024, Shalaby *et al*., 2022).

In this context, *Trichoderma*-based biological products represent a promising alternative for improving seedling quality during the nursery stage (Ferreira *et al*., 2024, Mwangi *et al*., 2013). *Trichoderma* species act as multifunctional agents by integrating biocontrol mechanisms and biostimulant effects associated with root colonization, the production of metabolites with hormone-like activity, nutrient solubilization, and the activation of physiological responses linked to stress tolerance (Contreras-Cornejo *et al*., 2024, Yao *et al*., 2023). These effects include the promotion of root development and vegetative growth, partially mediated by fungal metabolites with hormone-like activity, including indole compounds such as indole-3-acetic acid (Contreras-Cornejo *et al*., 2024, Nieto-Jacobo *et al*., 2017). Likewise, under abiotic stress conditions, *Trichoderma* spp. can promote osmotic adjustment, strengthen antioxidant capacity, and activate priming responses, allowing plants to respond more rapidly to subsequent stress events (dos Santos *et al*., 2025, Morán-Diez *et al*., 2021).

During acclimatization and nursery management, applications of *Trichoderma* spp. have shown favorable effects on banana seedling quality by improving morphophysiological traits such as plant height, pseudostem diameter, number of leaves, leaf area, and nutrient accumulation (Moreira *et al*., 2021, Sanó *et al*., 2022). Consistent with these effects on root development, fungal metabolites such as anthranilic acid produced by *T. guizhouense* have been associated with the formation of lateral roots and root hairs through auxin-like signaling pathways (Chen *et al*., 2024). The use of compost enriched with *Trichoderma* spp. in the acclimatization substrate has also promoted the growth of micropropagated banana seedlings (Eliyanti *et al*., 2023). Likewise, *Trichoderma* spp. bioproducts can modulate the rhizosphere microbiota and improve the uptake of nitrogen and other mineral nutrients, contributing to the production of more vigorous seedlings (Fiorentino *et al*., 2018, Shao *et al*., 2025).

Nevertheless, comparative evidence on the performance of different commercial *Trichoderma*-based formulations during the nursery stage of micropropagated banana seedlings remains limited, particularly under local production conditions. Therefore, this study aimed to evaluate the effect of three *Trichoderma*-based biological products on the growth and quality of banana seedlings under nursery conditions using morphoagronomic variables associated with early establishment.

MATERIALS AND METHODS

Research site

The experiment was conducted at "La Fortuna" farm, located in La Chontilla precinct, La Guaya parish, El Empalme canton, Guayas province, Ecuador. The experimental site is located at 1°00′21.2″ S and 79°37′27.8″ W, at 96 m a.s.l. The area has a humid tropical climate, with a mean annual temperature of 25.4 °C, mean annual precipitation of 2352.2 mm, and average relative humidity of 84%. These climatic data correspond to 2024 records from the National Institute of Meteorology and Hydrology of Ecuador (INAMHI, 2024).

Plant material

Ex vitro banana seedlings (*Musa paradisiaca* L.) cv. Williams, in phase IV of acclimatization and with an average initial height of 10 cm, were used. The plant material was obtained from the certified laboratory GALILTEC ECUADOR GLTC S.A. to ensure initial uniformity and phytosanitary quality of the seedlings.

Experimental design and treatments

The experiment was established under a completely randomized design, with five treatments and four replications per treatment. Each experimental unit consisted of one banana seedling planted in a substrate-filled nursery bag. Treatments were randomly assigned to the experimental units within the nursery to reduce possible effects associated with light, humidity, or temperature gradients. Three *Trichoderma* spp.-based biological products were evaluated: BIO-TRICH, Trichoplus, and Tricomix. The biological treatments were standardized to deliver the same microbial dose, equivalent to 1×10^8 CFU or viable propagules plant^{-1}, depending on the type of formulation and the declared microbial concentration of each product. In addition, a reference mineral fertilization treatment with YaraMila™ INTEGRADOR™ and an untreated control without biostimulant application were included. The evaluated treatments are described in Table 1.

Biological products

The doses of the *Trichoderma* spp.-based biological products were standardized according to the number of viable microbial propagules applied per plant, rather than by product mass or volume, because the evaluated formulations differed in physical form, microbial composition, and declared concentration. For all biological treatments, the target microbial dose was

Table 1. Treatments evaluated in banana seedlings under nursery conditions.

Treatment	Description	Main composition	Standardized biological dose
T1	BIO-TRICH	*Trichoderma harzianum*	1×10^8 CFU plant^{-1}
T2	Trichoplus	*Trichoderma spp.*	1×10^8 CFU plant^{-1}
T3	Tricomix	*Trichoderma viride* + *Trichoderma harzianum*	1×10^8 viable spores plant^{-1}
T4	YaraMila™ INTEGRADOR™	Reference mineral fertilizer	1 g plant^{-1}
T5	Untreated control	No biostimulant	Water

adjusted to 1×10^8 CFU or viable propagules plant^{-1}. BIO-TRICH is a solid powder formulation based on live spores of *Trichoderma harzianum*, with a minimum concentration of 1.0×10^9 CFU g^{-1}. Trichoplus is a liquid formulation based on *Trichoderma* spp., with a reported concentration of 1.0×10^9 CFU mL^{-1}. Tricomix is a solid formulation composed of *Trichoderma viride* and *Trichoderma harzianum*. For this product, the amount applied per plant was adjusted according to the declared spore concentration and the net weight of the commercial package to deliver 1×10^8 viable spores plant^{-1}. All biological products were suspended in water immediately before application and applied as a root-zone drench. The final application volume was standardized at 50 mL plant^{-1} for all treatments to ensure uniform delivery of the microbial dose to the rhizosphere. The untreated control received the same volume of water without biological product application. Applications were performed at transplanting and subsequently at 15, 30, 45, and 60 days after transplanting. The final evaluation was carried out at 70 days after transplanting.

Experimental management

Seedlings were transplanted into nursery bags previously filled with disinfected substrate. The substrate consisted of a 3:1 (v/v) mixture of agricultural topsoil and compost. This mixture was used to provide adequate physical structure, organic matter, nutrient availability, aeration, and drainage during the nursery stage. The physicochemical characterization of the substrate showed 11 ppm NH_4^+-N, 27 ppm P, 0.16 cmolc kg^{-1} K, 10.0 cmolc kg^{-1} Ca, 0.8 cmolc kg^{-1} Mg, 20 ppm S, 216 ppm Fe, 5.8 ppm Zn, and 5.0 ppm Mn, with a pH of 6.0 and 3.8% organic matter. Throughout the experiment, the substrate was maintained close to field capacity by irrigation every two days. Weed control was performed manually each week. The experimental units were individually labeled to ensure their identification throughout the experimental period. Morphoagronomic variables were recorded at 70 days after transplanting.

Evaluated variables

Plant height

Plant height was recorded at 70 days after transplanting and expressed in centimeters. Measurements were taken from the base of the pseudostem, at the substrate surface, to the V-shaped insertion point of the youngest fully expanded leaf, using a measuring tape (Stanley® Fatmax® Autolock 5 m, class II, ±0.5 mm at 1 m) (Hooks *et al*., 2008).

Absolute growth rate

The absolute growth rate in plant height (AGR) was expressed as cm week^{-1}. Initial and final plant height were recorded for each seedling. AGR was calculated as the difference between final and initial height divided by the evaluation interval, using the following equation:

$$AGR = \frac{Hf - Hi}{tf - ti}$$

where AGR is the absolute growth rate in plant height, *Hf* is the final plant height, *Hi* is the initial plant height, *tf* is the final evaluation time, and *ti* is the initial evaluation time. The term $tf - ti$ corresponds to the evaluation interval expressed in weeks (Radford, 1967).

Pseudostem diameter

Pseudostem diameter was recorded at 70 DAT and expressed in centimeters. Measurements were taken 2 cm above the substrate surface using a digital caliper (Mitutoyo® Absolute Digimatic, ±0.02 mm). For each seedling, one measurement was taken at the same fixed height, avoiding visible deformations and leaf insertion points (Mateus-Cagua and Rodriguez-Yzquierdo., 2019).

Leaf emission interval

Leaf emission interval (LEI) was expressed as weeks leaf^{-1}, according to the criterion used to estimate leaf emission dynamics in *Musa* spp. (Orjeda, 1998). The number of leaves present at transplanting and the final number of functional leaves at 70 DAT were recorded. Only visible and functional leaves were considered. LEI was calculated as the time required to emit one new leaf, using the following equation:

$$\text{LEI} = \frac{tf - ti}{NLf - NLi}$$

where LEI is the leaf emission interval, *NLf* is the final number of leaves, *NLi* is the initial number of leaves, and $tf - ti$ is the evaluation interval expressed in weeks. Lower LEI values indicate faster leaf emission.

Total leaf area

Total leaf area per plant was estimated using a non-destructive method based on linear measurements of each functional leaf. Leaf blade length was measured from the base of the lamina to the apex, and maximum width was measured at the middle section of each leaf. The individual area of each leaf was calculated using the following equation:

$$LAi = Li \times Wi \times 0.80$$

where LAi is the individual leaf area of leaf i, Li is the length of leaf i, Wi is the maximum width of leaf i, and 0.80 is the correction coefficient for banana leaves (Kumar *et al*., 2002). Subsequently, total leaf area per plant was obtained by summing the estimated area of all functional leaves present on each seedling:

$$TLA = \sum_{i=1}^{n} LAi$$

where TLA is the total leaf area per plant and n is the number of functional leaves evaluated on each seedling. Results were expressed as cm² plant⁻¹.

SPAD chlorophyll index

The chlorophyll index was estimated using a portable SPAD chlorophyll meter. For each seedling, four readings were taken from the middle section of the youngest fully expanded leaf, avoiding the midrib, leaf margins, and areas with visible damage. The final value per plant corresponded to the average of the four readings. Results were expressed as SPAD units.

Total root length

Total root length was determined at 70 days after transplanting. Seedlings were carefully removed from the nursery bags, and the root system was washed with water to remove adhering substrate while avoiding root breakage. Subsequently, the roots of each seedling were spread on a flat, contrasting surface, avoiding root overlap as much as possible. Each root system was photographed together with a metric scale placed on the same measurement plane. Images were captured perpendicular to the measurement surface to reduce distortion. The photographs were then calibrated in ImageJ using the metric scale included in each image (Cui *et al*., 2024). The length of each visible root was measured from its insertion point to the apex using the segmented line tool. Total root length per plant was obtained by summing the individual lengths of all measured roots, and results were expressed as cm plant⁻¹.

Fresh and dry biomass

Seedlings were carefully removed from the nursery bags; roots were washed with distilled water to remove adhering substrate, and excess surface moisture was removed with absorbent paper without compressing the tissue. Each plant was then separated into leaves, pseudostem, and roots using previously disinfected stainless-steel scissors. Fresh biomass (g plant⁻¹) of each fraction was determined immediately after fractionation using a digital balance (Sartorius Entris 2202-1S, Sartorius AG, Göttingen, Germany; ± 0.01 g). Total fresh biomass was calculated as the sum of the three fractions (Mateus-Cagua and Rodriguez-Yzquierdo, 2019).

For dry biomass determination (g plant⁻¹), each fraction was placed in labeled paper bags. Samples were dried in a forced-air oven at 65 °C for 72 h or until constant weight. After drying, samples were weighed using the same balance. Total dry biomass was calculated as the sum of the dry biomass of the three fractions.

Tissue water content

Tissue water content was calculated based on the total fresh mass of each seedling, following the criterion used to estimate the percentage of water in plant tissues from the difference between fresh and dry mass (Abdelaziz *et al*., 2014). The following equation was used:

$$TWC\ (\%) = \frac{FM - DM}{FM} \times 100$$

where TWC is the tissue water content, *FM* is the total fresh mass, and *DM* is the total dry mass of each seedling. Results were expressed as a percentage.

Root-to-shoot ratio

The root-to-shoot ratio was calculated from root dry biomass and shoot dry biomass of each seedling, following the criterion based on the ratio between root dry biomass and shoot dry biomass (Santangeli *et al*., 2024). Shoot dry biomass corresponded to the sum of leaf and pseudostem dry biomass. The ratio was calculated using the following equation:

$$RSR = \frac{RDB}{SDB}$$

where RSR is the root-to-shoot ratio, *RDB* is root dry biomass, and *SDB* is shoot dry biomass.

Specific leaf area

Specific leaf area was calculated as the ratio between total leaf area and leaf dry biomass of each seedling, following the criterion of leaf area per unit of leaf dry mass (Wang *et al*., 2019). The following equation was used:

$$SLA = \frac{TLA}{LDB}$$

where SLA is the specific leaf area, *TLA* is the total leaf area per plant, and *LDB* is leaf dry biomass. Results were expressed as cm² g⁻¹.

Specific root length

Specific root length was calculated from the total root length and root dry biomass of each seedling, following the criterion used to estimate root length per unit of root dry biomass (Santangeli *et al*., 2024). The following equation was used:

$$SRL = \frac{TRL}{RDB}$$

where *SRL* is the specific root length, *TRL* is the total root length, and *RDB* is root dry biomass. Results were expressed as cm g^{-1}.

Slenderness index

The slenderness index was calculated as the ratio between plant height and pseudostem diameter, following the height-to-diameter criterion used as a non-destructive morphological indicator of seedling quality under nursery conditions. Plant height was expressed in centimeters, whereas pseudostem diameter was expressed in millimeters (Gallegos-Cedillo *et al*., 2021). The index was calculated using the following equation:

$$SI = \frac{H}{PD}$$

where SI is the slenderness index, *H* is plant height expressed in centimeters, and *PD* is pseudostem diameter expressed in millimeters. Results were expressed as cm mm^{-1}.

Dickson quality index

The Dickson quality index was calculated from total dry biomass, the plant height-to-pseudostem diameter ratio, and the shoot-to-root dry biomass ratio, following the equation used to estimate the morphological quality of seedlings (Dickson *et al*., 1960). The Dickson quality index was calculated using the following equation:

$$DQI = \frac{TDB}{\left(\frac{H}{PD}\right) + \left(\frac{SDB}{RDB}\right)}$$

where DQI is the Dickson quality index, *TDB* is total dry biomass, *H* is plant height, *PD* is pseudostem diameter, *SDB* is shoot dry biomass, and *RDB* is root dry biomass. Results were expressed in grams.

Statistical analysis

All statistical analyses were performed in R version 4.4.2. Data were analyzed considering treatment as a fixed factor and replication as the experimental unit. When subsamples were present within a replication, they were averaged beforehand to avoid pseudoreplication. Results were expressed as mean ± standard deviation. The normality of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Variables that met the assumptions for parametric analysis were analyzed using one-way ANOVA, followed by Tukey's test for mean comparison ($p \leq 0.05$). When heterogeneity of variances was detected, Welch's ANOVA was applied; when significant effects were found, multiple comparisons were performed using the Games–Howell test. Variables that did not meet the assumptions for parametric analysis were analyzed using the Kruskal–Wallis test and, when appropriate, Dunn's test with Holm adjustment. In addition, principal component analysis (PCA) was performed using standardized variables through z-score transformation to explore the multivariate association between treatments and seedling quality variables. Statistical significance was set at $p \leq 0.05$.

RESULTS

Vegetative growth of banana seedlings

The evaluated treatments significantly affected the vegetative growth variables of banana seedlings under nursery conditions ($p \leq 0.05$; Figure 1). The untreated control showed the lowest values for growth rate, plant height, pseudostem diameter, and leaf area, but the highest leaf emission interval. Growth rate was significantly higher in T1, T2, and T3 than in the YaraMila™ INTEGRADOR™ treatment and the untreated control ($p \leq 0.05$). BIO-TRICH, Trichoplus, and Tricomix reached growth rates of 2.75, 2.68, and 2.62 cm $week^{-1}$, respectively, exceeding the untreated control by 186.5 %, 179.2%, and 172.9%, respectively. Compared with YaraMila™ INTEGRADOR™, these treatments increased growth rate by 26.1%, 22.9%, and 20.2%, respectively (Figure 1A). For plant height, the *Trichoderma* spp. treatments reached values ranging from 23.75 to 24.67 cm, with no significant differences among them ($p > 0.05$), but they were significantly higher than the YaraMila™ INTEGRADOR™ reference treatment and the untreated control ($p \leq 0.05$). Relative to the untreated control, BIO-TRICH, Trichoplus, and Tricomix increased plant height by 107.3%, 107.7%, and 99.9%, respectively (Figure 1B).

Pseudostem diameter was also enhanced by the biological treatments. BIO-TRICH, Trichoplus, and Tricomix showed diameters of 5.63, 5.51, and 5.57 cm, respectively, with no significant differences among them ($p > 0.05$). These values were significantly higher than those obtained with YaraMila™ INTEGRADOR™ and the untreated control ($p \leq 0.05$). Compared with the untreated control, the *Trichoderma* spp. treatments increased pseudostem diameter by 65.0% to 68.6%; relative to the YaraMila™ INTEGRADOR™ treatment, the increase ranged from 14.6% to 17.0% (Figure 1C). Leaf emission interval was also significantly affected by the treatments. The untreated control showed the highest value, with approximately 1.65 weeks leaf^{-1}, indicating a longer time required to emit a new leaf. In contrast, Tricomix recorded the lowest value, with approximately 1.04 weeks leaf^{-1}, followed by BIO-TRICH and Trichoplus, with values close to 1.12- and 1.18-weeks leaf^{-1}, respectively. These results indicate that the *Trichoderma* spp.-based treatments reduced the time required for leaf emission, reflecting faster leaf production under nursery conditions (Figure 1D). Total leaf area showed a marked response to the treatments. BIO-TRICH, Trichoplus, and Tricomix reached 978.43, 922.84, and 969.41 cm² plant^{-1}, respectively, with no significant differences among them ($p > 0.05$), but with values significantly higher than those of the YaraMila™ INTEGRADOR™ treatment and the untreated control ($p \leq 0.05$) (Figure 1E). Overall, the *Trichoderma* spp.-based formulations promoted greater vegetative growth than both the reference mineral treatment and the untreated control.

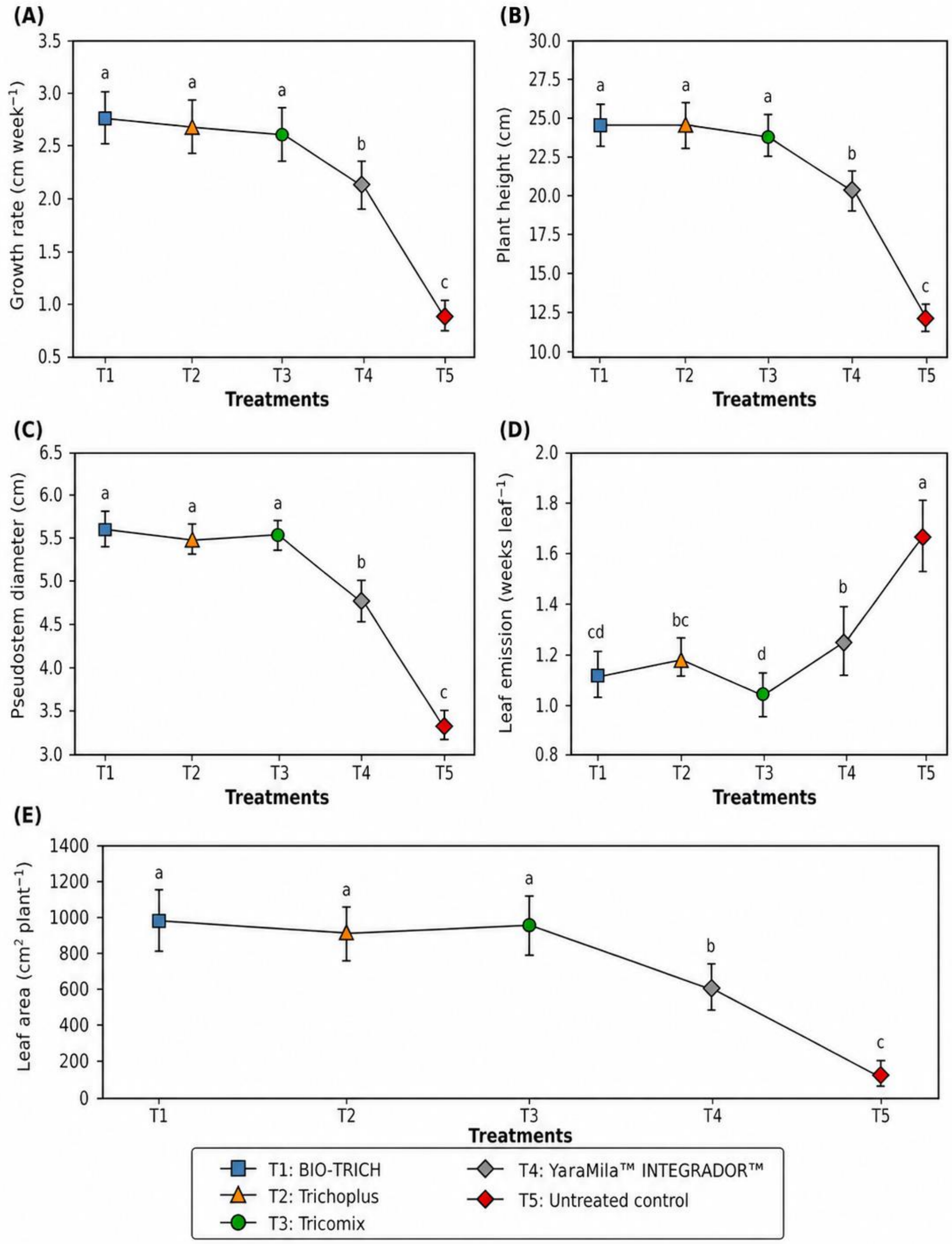

Figure 1. Effect of *Trichoderma* spp.-based biostimulants on the vegetative growth of banana seedlings under nursery conditions. (A) Absolute growth rate, (B) plant height, (C) pseudostem diameter, (D) leaf emission interval, and (E) total leaf area. Values are presented as mean ± standard deviation. Different letters indicate significant differences among treatments according to Tukey' s test (p ≤ 0.05).

Biomass accumulation and partitioning in banana seedlings

The *Trichoderma* spp.-based biostimulant treatments promoted greater fresh biomass accumulation in roots, leaves, pseudostem, and total biomass, whereas the untreated control showed the lowest values. Root fresh biomass differed significantly among treatments ($p \leq 0.05$). BIO-TRICH and Tricomix recorded the highest values, with 20.57 and 21.38 g plant^{-1}, respectively, with no significant differences between them ($p > 0.05$). Compared with the untreated control, these treatments increased root fresh biomass by 114.9% and 123.4%, respectively. Trichoplus and YaraMila™ INTEGRADOR™ did not differ significantly from either the highest-performing treatments or the untreated control ($p > 0.05$) (Figure 2A). In leaves, the *Trichoderma* spp. treatments reached the highest fresh biomass values, with no significant differences among BIO-TRICH, Trichoplus, and Tricomix ($p > 0.05$). These treatments were significantly superior to YaraMila™ INTEGRADOR™ and the untreated control ($p \leq 0.05$). Compared with the untreated control, BIO-TRICH, Trichoplus, and Tricomix increased leaf fresh biomass by 245.3%, 256.0%, and 307.9%, respectively. Relative to the reference mineral treatment, the increases were 62.8%, 67.8%, and 92.3%, respectively (Figure 2B).

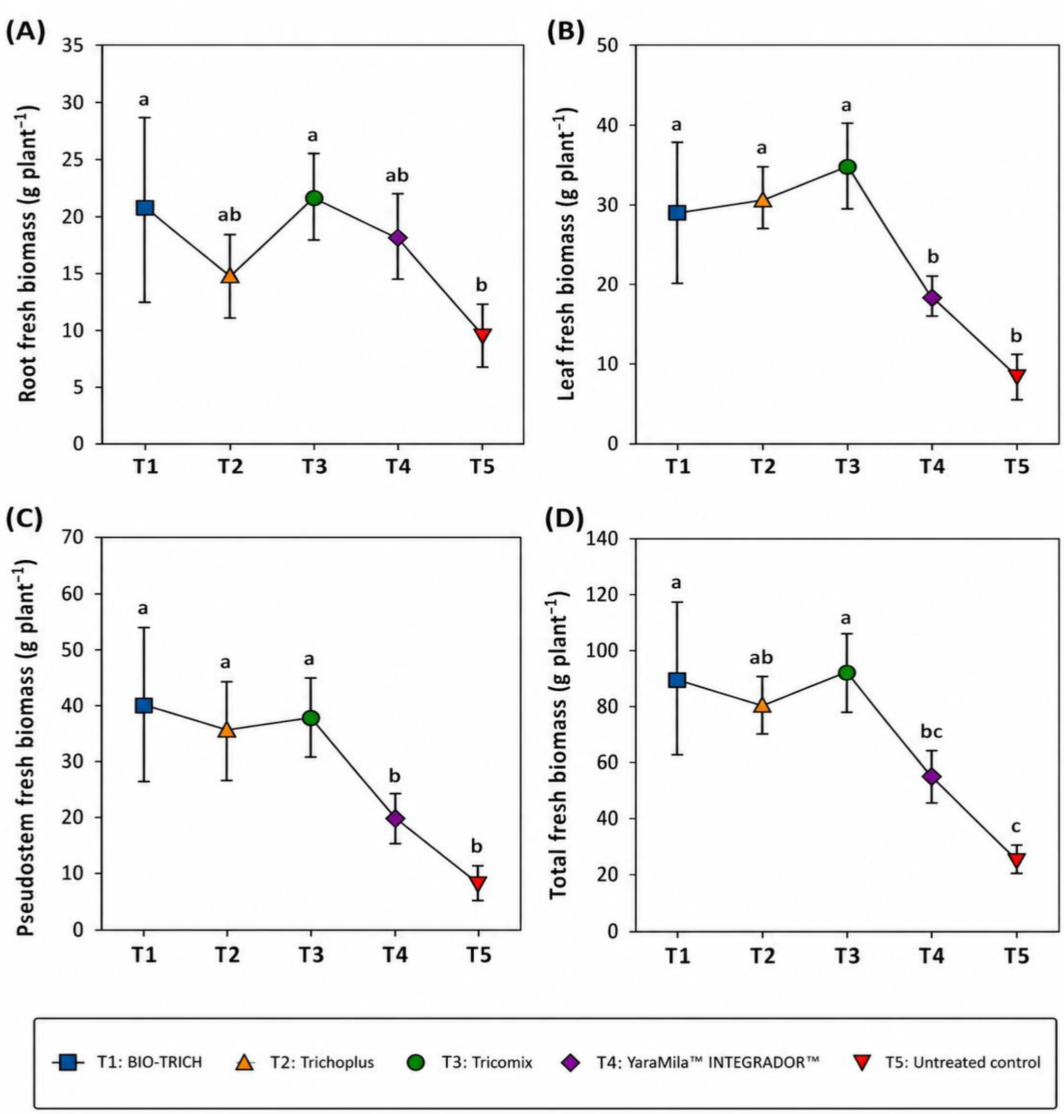

Figure 2. Effect of *Trichoderma* spp.-based biostimulants on fresh biomass accumulation by organ in banana seedlings under nursery conditions. Root fresh biomass (A), leaf fresh biomass (B), pseudostem fresh biomass (C), and total fresh biomass (D). Values are presented as mean ± standard deviation. Different letters indicate significant differences among treatments according to Tukey's test (p ≤ 0.05).

Pseudostem fresh biomass showed a similar response. The biological treatments did not differ significantly from one another ($p > 0.05$) and were significantly higher than YaraMila™ INTEGRADOR™ and the untreated control ($p \leq 0.05$). Relative to the untreated control, BIO-TRICH, Trichoplus, and Tricomix increased pseudostem fresh biomass by 382.1%, 330.6%, and 358.9%, respectively (Figure 2C). Total fresh biomass was also significantly affected by the treatments ($p \leq 0.05$). Tricomix and BIO-TRICH showed the highest values, with 93.92 and 89.75 g plant^{-1}, respectively, with no significant differences between them or compared with Trichoplus ($p > 0.05$). Compared with the untreated control, the *Trichoderma* spp. treatments increased total fresh biomass by 241.0% for BIO-TRICH, 206.6% for Trichoplus, and 256.8% for Tricomix (Figure 2D).

The evaluated treatments significantly modified dry biomass accumulation and partitioning in banana seedlings under nursery conditions ($p \leq 0.05$; Figure 3). Root dry biomass differed significantly among treatments ($p \leq 0.05$). Tricomix recorded the highest value, with 1.33 g plant^{-1}, and was significantly higher than Trichoplus and the untreated control. Compared with the untreated control, Tricomix increased root dry biomass by 121.7%, whereas BIO-TRICH and YaraMila™ INTEGRADOR™ increased it by 71.7% and 83.3%, respectively. However, BIO-TRICH did not differ significantly from the other treatments ($p > 0.05$) (Figure 3A). Leaf dry biomass was also significantly affected by the treatments ($p \leq 0.05$). BIO-TRICH, Trichoplus, and Tricomix showed the highest values, with 2.90, 2.87, and 3.30 g plant^{-1}, respectively, with no significant differences among them ($p > 0.05$). These treatments were significantly higher than YaraMila™ INTEGRADOR™ and the untreated control ($p \leq 0.05$). Compared with the untreated control, the *Trichoderma* spp. treatments increased leaf dry biomass by 211.8% for BIO-TRICH, 208.6% for Trichoplus, and 254.8% for Tricomix. Relative to the reference mineral treatment, the increases were 55.1%, 53.5%, and 76.5%, respectively (Figure 3B).

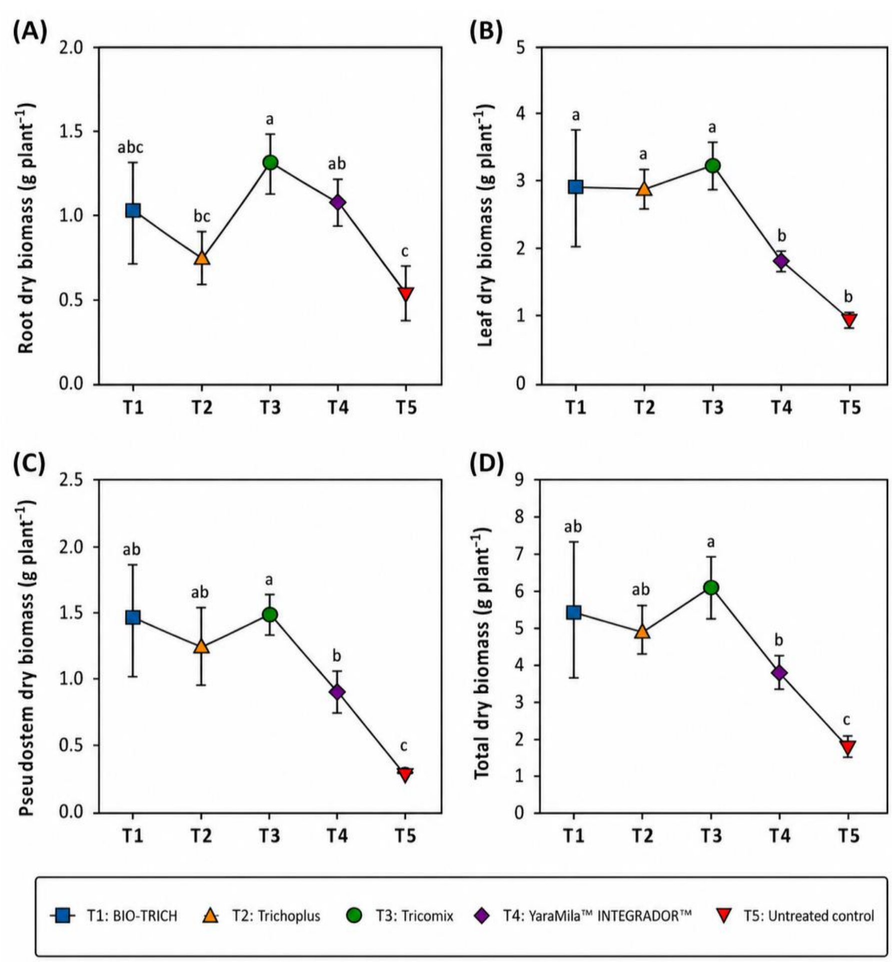

Figure 3. Effect of *Trichoderma* spp.-based biostimulants on dry biomass accumulation by organ in banana seedlings under nursery conditions. Root dry biomass (A), leaf dry biomass (B), pseudostem dry biomass (C), and total dry biomass (D). Values are presented as mean ± standard deviation. Different letters indicate significant differences among treatments according to Tukey's test (p ≤ 0.05).

For pseudostem dry biomass, Tricomix reached the highest value, with 1.50 g plant⁻¹, and was significantly higher than YaraMila™ INTEGRADOR™ and the untreated control ($p \leq 0.05$). BIO-TRICH and Trichoplus showed intermediate values, without differing significantly from Tricomix or YaraMila™ INTEGRADOR™ ($p > 0.05$), but they were significantly higher than the untreated control. BIO-TRICH, Trichoplus, and Tricomix increased pseudostem dry biomass by 390.0%, 310.0%, and 400.0%, respectively, compared with the untreated control (Figure 3C). Total dry biomass was significantly higher in the treatments with the best biological performance ($p \leq 0.05$). Tricomix showed the highest value, with 6.13 g plant^{-1}, followed by BIO-TRICH and Trichoplus, with 5.40 and 4.87 g plant^{-1}, respectively. These treatments did not differ significantly from one another ($p > 0.05$), although Tricomix was significantly higher than YaraMila™ INTEGRADOR™ and the untreated control. Compared with the untreated control, BIO-TRICH, Trichoplus, and Tricomix increased total dry biomass by 195.1%, 166.1%, and 235.0%, respectively. Relative to YaraMila™ INTEGRADOR™, the increases were 39.5%, 25.8%, and 58.4%, respectively (Figure 3D).

Physiological status and tissue water content

The treatments significantly affected the SPAD chlorophyll index of banana seedlings under nursery conditions ($p \leq 0.05$; Figure 4A). The *Trichoderma* spp.-based biostimulant treatments showed the highest values, with no significant differences among BIO-TRICH, Trichoplus, and Tricomix ($p > 0.05$). Tricomix reached the highest SPAD index, with 55.10 units, followed by Trichoplus (53.45 units) and BIO-TRICH (52.90 units). These three treatments were significantly higher than the YaraMila™ INTEGRADOR™ treatment and the untreated control ($p \leq 0.05$). Compared with the untreated control, BIO-TRICH, Trichoplus, and Tricomix increased the chlorophyll index by 45.0%, 46.5%, and 51.0%, respectively. In contrast, tissue water content did not differ significantly among treatments ($p > 0.05$; Figure 4B). Values ranged from 92.94% to 94.00%, with a slight trend toward higher contents in BIO-TRICH and Trichoplus; however, these differences were not statistically significant.

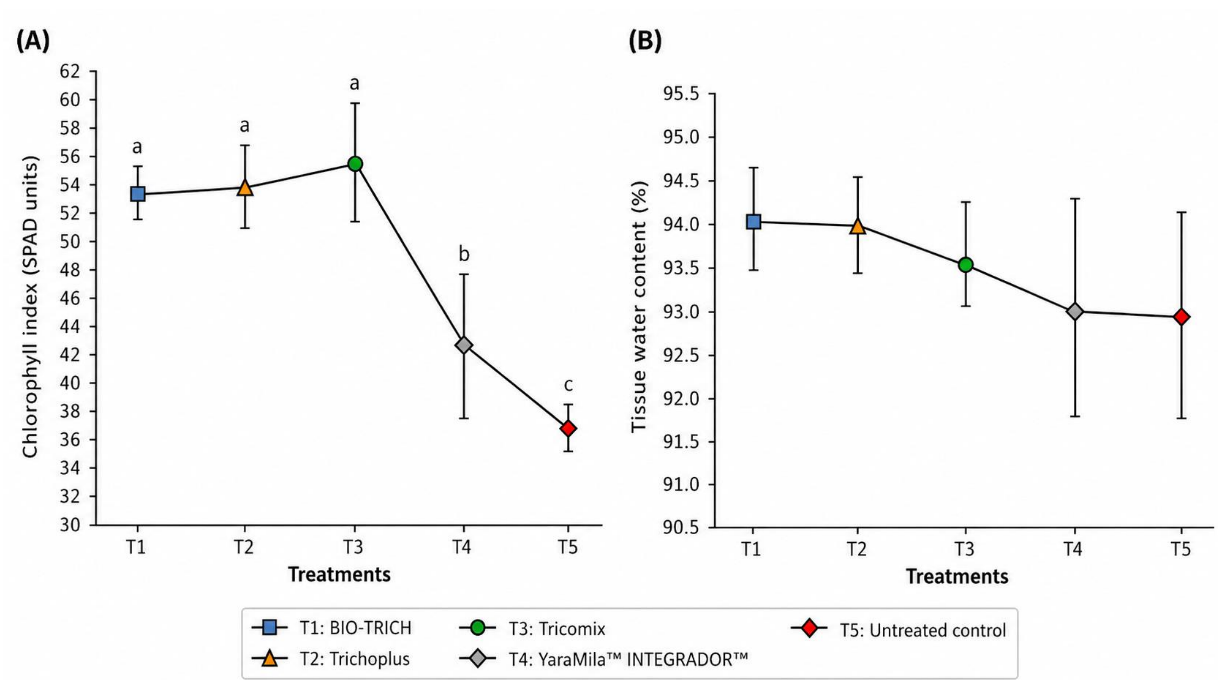

Figure 4. Effect of the treatments on the SPAD chlorophyll index (A) and tissue water content (B) in banana seedlings under nursery conditions. Values are presented as mean ± standard deviation. In panel A, different letters indicate significant differences among treatments according to Tukey's test ($p \leq 0.05$). In panel B, letters are not shown because no significant differences were detected among treatments.

Root traits and seedling quality indices

Total root length did not differ significantly among treatments ($p > 0.05$; Figure 5A), although Tricomix showed the highest numerical value, with 34.26 cm plant^{-1}, exceeding the untreated control by 32.8%. The root-to-shoot ratio was significantly affected by the treatments ($p \leq 0.05$; Figure 5B). The untreated control showed the highest value (0.49), followed by YaraMila ™ INTEGRADOR™ (0.40) and Tricomix (0.28). Specific root length did not differ significantly among treatments ($p > 0.05$; Figure 5C). The untreated control showed the highest numerical value (43.64 cm g^{-1}), followed by Trichoplus (38.46 cm g^{-1}) and BIO-TRICH (33.22 cm g^{-1}). Specific leaf area also did not differ significantly among treatments ($p > 0.05$; Figure 5D). However, BIO-TRICH showed the highest numerical value (367.17 cm² g^{-1}), followed by YaraMila™ INTEGRADOR™ (344.59 cm² g^{-1}) and Trichoplus (325.00 cm² g^{-1}). Compared with the untreated control, BIO-TRICH, Trichoplus, and Tricomix showed numerical increases of 100.2%, 77.2%, and 63.2%, respectively; however, these differences were not statistically significant.

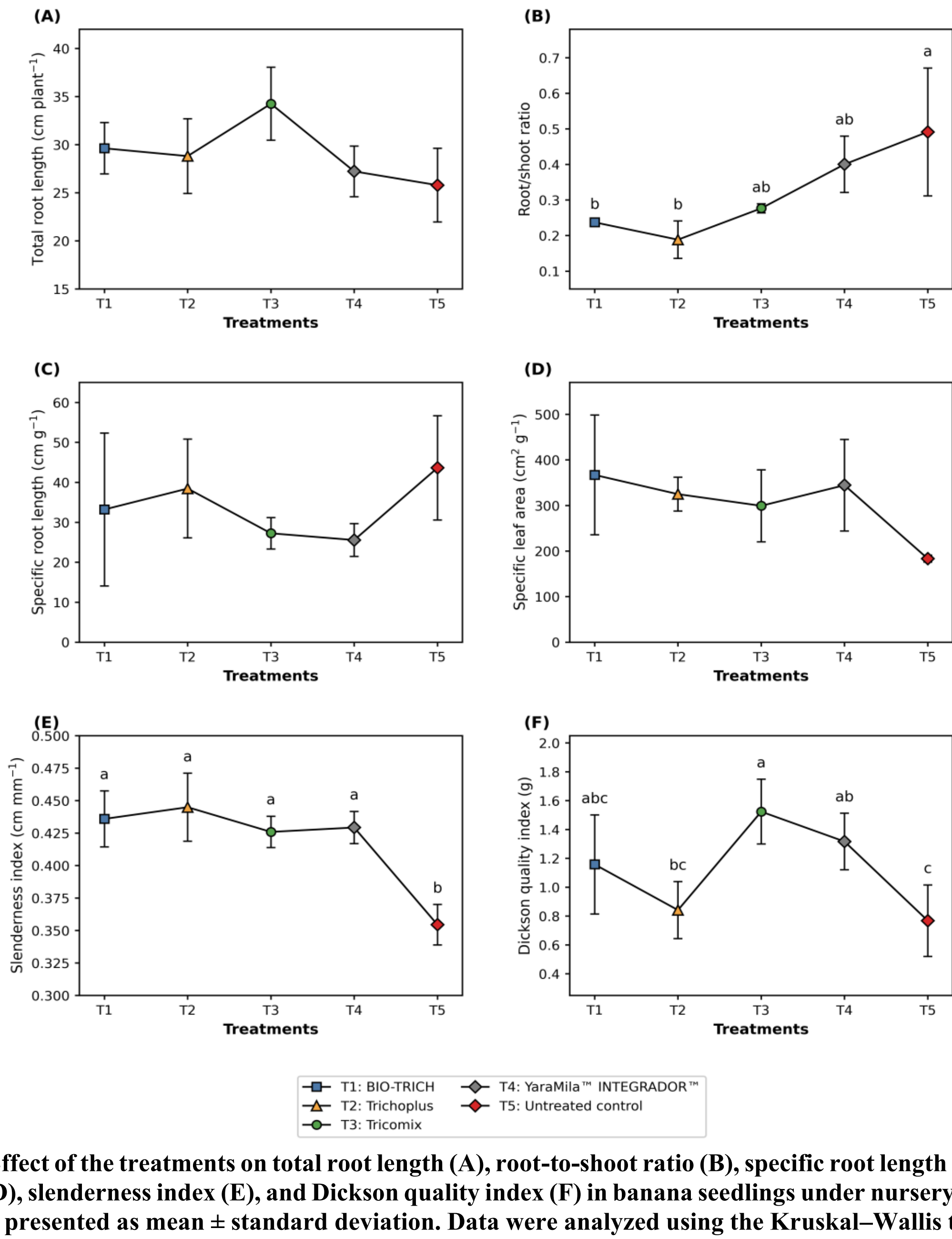

Figure 5. Effect of the treatments on total root length (A), root-to-shoot ratio (B), specific root length (C), specific leaf area (D), slenderness index (E), and Dickson quality index (F) in banana seedlings under nursery conditions. Values are presented as mean ± standard deviation. Data were analyzed using the Kruskal–Wallis test in panel A, one-way ANOVA followed by Tukey's test in panels B, E, and F, Welch's ANOVA in panel C, and one-way ANOVA in panel D. Different letters indicate significant differences among treatments (p ≤ 0.05). In panels A, C, and D, letters are not shown because no significant differences were detected among treatments.

The slenderness index differed significantly among treatments ($p \leq 0.05$; Figure 5E). BIO-TRICH, Trichoplus, Tricomix, and YaraMila™ INTEGRADOR™ did not differ significantly from one another ($p > 0.05$) and showed values ranging from 0.43 to 0.44 cm mm^{-1}. In contrast, the untreated control showed the lowest value (0.35 cm mm^{-1}) and differed significantly from the other treatments. The Dickson quality index was significantly affected by the treatments ($p \leq 0.05$; Figure 5F). Tricomix showed the highest value (1.52 g), followed by YaraMila™ INTEGRADOR™ (1.32 g) and BIO-TRICH (1.16 g). Compared with the untreated control, Tricomix increased the Dickson quality index by 98.4%, YaraMila™ INTEGRADOR™ by 71.4%, and BIO-TRICH by 50.8%. Trichoplus showed a smaller increase (9.6%) and did not differ significantly from the untreated control. These results indicate that Tricomix produced seedlings with greater integrated morphological quality by combining greater biomass accumulation, a more favorable height-to-diameter relationship, and a better balance between shoot and root biomass.

Multivariate analysis of seedling quality variables

The first two principal components jointly explained 80.7% of the total variation, with 60.7% explained by PC1 and 20.0% by PC2, indicating that the biplot adequately summarized most of the variability observed among treatments. PC1 mainly represented a gradient of morphological vigor and biomass accumulation. On the negative side of this component, variables associated with greater growth and seedling quality were grouped, including H—plant height—, PD—pseudostem diameter—, TLA—total leaf area—, TRL—total root length—, TDB—total dry biomass—, SI—slenderness index—, and DQI—Dickson quality index—. In contrast, the positive side of PC1 was mainly associated with RSR—root-to-shoot ratio—, toward which the experimental units of the untreated control were displaced. The *Trichoderma* spp.-based biostimulant treatments were mostly distributed toward the negative side of PC1, confirming their association with greater vegetative growth, biomass accumulation, and morphological quality. In particular, T3 (Tricomix) was located close to the TDB and DQI vectors, which is consistent with its higher total dry biomass and higher Dickson quality index. T1 (BIO-TRICH) was also associated with the group of growth and biomass variables, although it showed greater dispersion among replications. T2 (Trichoplus) was mainly located in the upper-left quadrant, closer to SLA—specific leaf area—, suggesting a greater relative contribution of this variable to its multivariate response. Treatment T4 (YaraMila™ INTEGRADOR™) showed an intermediate distribution, with experimental units located near the origin or toward the lower quadrant, indicating a less marked morphological response than that observed in the biological treatments. T5, the untreated control, was clearly separated from the other treatments toward the positive side of PC1. This separation was mainly associated with RSR and, to a lesser extent, with SRL—specific root length—. However, this association should not be interpreted as better seedling quality, because the untreated control showed the lowest values for plant height, pseudostem diameter, leaf area, total dry biomass, and Dickson quality index. Therefore, its higher root-to-shoot ratio mainly reflects lower shoot biomass accumulation rather than a functional improvement of the root system. Overall, the PCA confirmed that the *Trichoderma* spp. formulations, particularly Tricomix, were associated with better morphological performance and integrative seedling quality indices.

DISCUSSION

The results of this study show that *Trichoderma* spp.-based formulations consistently improved vegetative growth, biomass accumulation, chlorophyll index, and several integrative quality indicators of banana seedlings under nursery conditions. This response confirms the role of *Trichoderma* spp. as a rhizosphere biostimulant, beyond its classical function as a biocontrol agent. Previous studies have indicated that the application of *Trichoderma* during the acclimatization of micropropagated banana seedlings is a relevant strategy to improve early growth and *ex vitro* adaptation, a stage in which plants are particularly sensitive because of their transition from *in vitro* conditions to an environment with greater water, light, and nutritional demand (Ramírez-Torres *et al*., 2023, Sanó *et al*., 2022).

The increase observed in growth rate, plant height, pseudostem diameter, leaf emission interval, and total leaf area in the BIO-TRICH, Trichoplus, and Tricomix treatments may be explained by the ability of *Trichoderma* spp. to colonize the rhizosphere and favorably modify plant–microorganism interactions. Growth promotion by *Trichoderma* has been associated with the production of metabolites with hormone-like activity, including indole compounds such as indole-3-acetic acid, as well as volatile organic compounds and fungal signals that can modulate endogenous plant growth pathways (Deel *et al*., 2024, Nieto-Jacobo *et al*., 2017). These mechanisms promote cell expansion, root development, nutrient uptake, and consequently, shoot growth (Contreras-Cornejo *et al*., 2009, 2024).

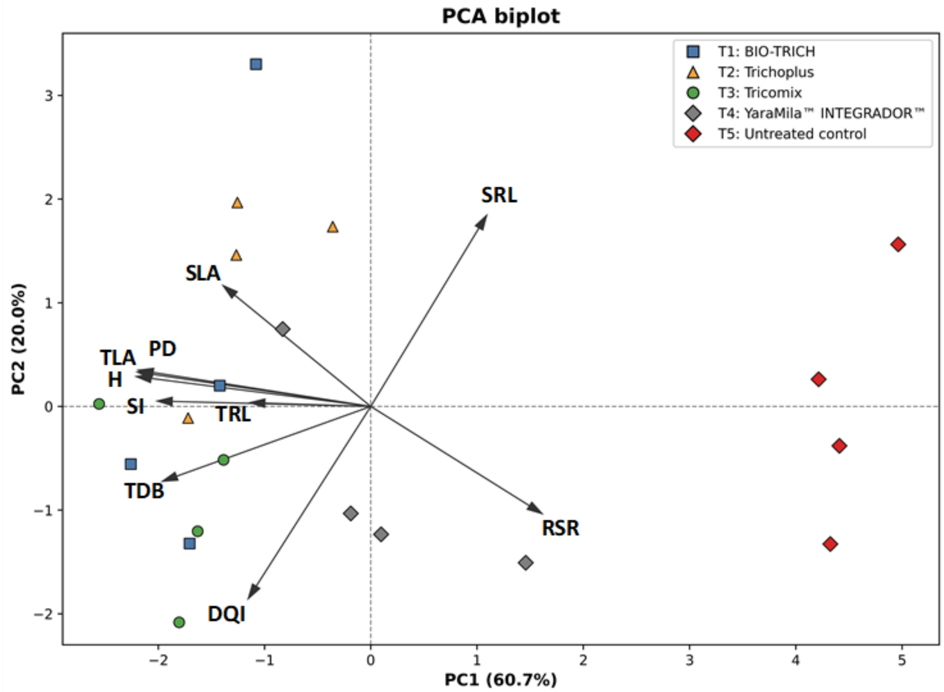

Figure 6. Principal component analysis (PCA) of morphological and quality variables in banana seedlings under different treatments under nursery conditions. Symbols represent the experimental units corresponding to T1: BIO-TRICH, T2: Trichoplus, T3: Tricomix, T4: YaraMila™ INTEGRADOR™, and T5: untreated control. Arrows indicate the contribution and direction of association of the variables with the first two principal components. PC1 explained 60.7% of the total variation and PC2 explained 20.0%. Abbreviations: H, plant height; PD, pseudostem diameter; TLA, total leaf area; TRL, total root length; TDB, total dry biomass; RSR, root-to-shoot ratio; SLA, specific leaf area; SRL, specific root length; SI, slenderness index; and DQI, Dickson quality index.

The superiority of the *Trichoderma* spp. treatments over the untreated control and the reference mineral treatment indicates that the response did not depend solely on external nutrient supply, but also on biological processes associated with biostimulation. Moreira *et al*. (2021) evaluated the interaction between organic substrates and inoculation with *Bacillus* sp. and *Trichoderma asperellum* in banana, reporting improvements in variables such as plant height, pseudostem diameter, leaf area, dry biomass, and physiological parameters, which supports the importance of beneficial microorganisms in banana seedling quality. Similar results were reported by Eliyanti *et al*. (2023), who demonstrated that compost enriched with *Trichoderma* spp. promoted the growth of banana seedlings during acclimatization, with significant effects on plant height, number of leaves, and leaf length. The shorter leaf emission interval recorded in the *Trichoderma* spp. treatments, particularly in Tricomix, suggests a faster leaf production pattern and a more dynamic vegetative growth response under nursery conditions. In banana, leaf emission is closely related to nutritional status, meristematic activity, and photosynthetic capacity (Meghwal *et al*., 2021, Turner *et al*., 2007). Therefore, a higher leaf production rate indicates a favorable response of the vegetative system.

The greater accumulation of dry biomass in leaves and pseudostem suggests higher photosynthetic activity and improved allocation of photoassimilates to structural organs. In banana seedlings, the pseudostem, which is composed of overlapping leaf sheaths, performs an important structural function as the support axis of the shoot and participates in the temporary storage of water, nutrients, and organic compounds; therefore, greater pseudostem development contributes to the morphological robustness of the plant during field establishment (Lima *et al*., 2020, Turner *et al*., 2007). In banana acclimatization studies, seedlings grown under more favorable conditions showed greater plant height, pseudostem diameter, root length, and fresh and dry biomass accumulation, which are considered favorable traits for the transition to field conditions (Melo *et al*., 2024, Rodrigues *et al*., 2022).

The intermediate response of the YaraMila™ INTEGRADOR™ treatment indicates that mineral fertilization partially improved growth compared with the untreated control, but did not reach the effect observed with the *Trichoderma* spp. formulations. This may be explained by the fact that mineral fertilization provides available nutrients but does not necessarily actively modify root architecture, the rhizosphere microbiota, or hormonal growth pathways. In contrast, *Trichoderma* can act through multiple mechanisms, including nutrient solubilization and mobilization, production of bioactive metabolites, root stimulation, and activation of beneficial physiological responses (Guzmán-Guzmán *et al*., 2025, Yao *et al*., 2023).

The increase in the SPAD index in the *Trichoderma* spp. treatments indicates an improvement in foliar physiological status. Although SPAD does not directly measure absolute chlorophyll concentration, it is widely used as a relative indicator of leaf greenness and is associated with photosynthetic capacity and nutritional status, particularly in relation to nutrients involved in chlorophyll metabolism. In banana seedlings, photosynthetic pigments are relevant during acclimatization because they sustain photosynthetic capacity and the production of photoassimilates required for growth, survival, and ex vitro hardening (Aremu *et al*., 2012, Shalaby *et al*., 2022).

The higher SPAD response in Tricomix, BIO-TRICH, and Trichoplus may be associated with greater nutrient uptake or nutrient-use efficiency, as well as improved root functionality. Moreira *et al*. (2021) reported that inoculation with *Trichoderma asperellum*, in interaction with the substrate, influenced the morphophysiological performance and nutritional content of banana seedlings, which is consistent with the physiological improvement observed in this study. In addition, the ability of *Trichoderma* spp. to induce biochemical changes in the plant metabolic profile, including compounds related to carbohydrate, amino acid, organic acid, and lipid metabolism, may contribute to an improved physiological status of the plant (Lombardi *et al*., 2020, Lotito *et al*., 2024, Senizza *et al*., 2023).

Total root length did not differ significantly among treatments, although Tricomix showed the highest numerical value. This lack of significance may be due to the inherent variability of the root system and to the fact that total root length alone does not always reflect root functionality, since only a fraction of the root system may actively participate in water and nutrient uptake (Cochavi *et al*., 2020, Huang *et al*., 2023, Kou *et al*., 2022). The root-to-shoot ratio was higher in the untreated control; however, this response should not be interpreted as an improvement in seedling quality. In this case, the higher value was mainly explained by lower shoot biomass rather than greater root biomass accumulation. A high root-to-shoot ratio may be positive when it reflects efficient root investment, but it may also result from restricted shoot growth. Because the untreated control showed lower values for plant height, pseudostem diameter, leaf area, biomass, and SPAD index, its higher root-to-shoot ratio represents an imbalance associated with low vigor (Poorter *et al*., 2012, Seidel *et al*., 2024).

Specific root length and specific leaf area did not differ significantly among treatments. Functionally, these variables reflect organ construction strategies: specific root length indicates root length produced per unit of root biomass, whereas specific leaf area indicates leaf area per unit of leaf dry biomass (Dawson *et al*., 2024, Pérez-Harguindeguy *et al*., 2013). The absence of differences suggests that the treatments mainly modified the magnitude of growth and biomass accumulation, rather than the specific morphological efficiency of root or leaf construction (Freschet *et al*., 2015, Ning *et al*., 2022). This is consistent with the biomass and leaf area results, where *Trichoderma* treatments increased seedling size and vigor but did not necessarily alter the structural relationship between area, length, and dry mass. The slenderness index was lower in the untreated control, but this reduction should also not be interpreted as greater robustness. In vigorous seedlings, a lower slenderness index may indicate a better balance between height and diameter; however, when this low value occurs together with simultaneous reductions in height, diameter, and biomass, it should be interpreted as an expression of restricted growth rather than greater morphological quality (Gallegos-Cedillo *et al*., 2021, Haase, 2008).

Regarding the Dickson quality index, Tricomix showed the highest value, followed by YaraMila™ INTEGRADOR™ and BIO-TRICH. This result indicates that Tricomix produced seedlings with a better balance among total biomass, height-to-diameter ratio, and shoot-to-root biomass ratio. The stronger response of Tricomix may be related to the combination of T. viride and T. harzianum, species that may express complementary functional traits, such as phosphate and micronutrient solubilization, siderophore production, indole-3-acetic acid synthesis, and enzymatic activity (Alwadai *et al*., 2024, Bader *et al*., 2020). These traits could expand the ability of the inoculant to favor rhizosphere colonization, nutrient mobilization, and plant growth promotion (Jamil, 2021, Li *et al*., 2015, Woo *et al*., 2023). Although the present study did not evaluate root colonization or fungal metabolites, the literature supports that plant–*Trichoderma* interactions can activate growth and stress tolerance responses through root colonization, hormonal signaling, antioxidant activation, and physiological reprogramming of the plant (Brotman *et al*., 2013, Dutta *et al*., 2023).

Principal component analysis confirmed the pattern observed in the univariate analyses. The *Trichoderma* spp. treatments were mainly associated with growth and quality variables, such as plant height, pseudostem diameter, total leaf area, total dry biomass, and Dickson quality index. This association indicates that the seedling response was not limited to an isolated variable but corresponded to an integrated pattern of greater morphological vigor. The separation of the untreated control toward the side associated with root-to-shoot ratio and specific root length confirms that these variables, when interpreted in isolation, may suggest an apparent improvement in root performance. However, when integrated with biomass, leaf area, plant height, and the Dickson quality index, the untreated control represents a lower-growth profile. The intermediate position of YaraMila™ INTEGRADOR™ suggests that mineral nutrition partially improved seedling performance but did not fully reproduce the effect of the biostimulants. This reinforces the idea that nursery seedling quality depends not only on nutrient availability, but also on rhizosphere functionality and the ability of beneficial microorganisms to modulate physiological and morphological processes. In this regard, the results are consistent with evidence showing that plant growth-promoting microorganisms can serve as multifunctional allies for improving plant performance under stress conditions or during critical establishment stages (Santoyo *et al*., 2024).

CONCLUSIONS

Trichoderma spp.-based bioproducts improved the morphophysiological quality of banana seedlings under nursery conditions by increasing vegetative growth, leaf area, biomass, and SPAD index compared with both the untreated control and the reference mineral treatment, YaraMila™ INTEGRADOR™. Tricomix was the formulation with the strongest response in terms of total dry biomass, Dickson quality index, and SPAD index. Therefore, its effect was not limited to increasing plant size but also promoted better integrated morphological quality. BIO-TRICH and Trichoplus also promoted seedling growth, particularly plant height, pseudostem diameter, growth rate, leaf area, and leaf biomass; however, their responses were less consistent than that observed with Tricomix for the integrative quality indicators. Total root length, specific root length, specific leaf area, and tissue water content did not differ significantly among treatments, indicating that the observed improvements were mainly related to biomass accumulation, vegetative vigor, and chlorophyll status rather than changes in the specific construction efficiency of roots or leaves. Overall, the results support the use of *Trichoderma* spp.-based formulations, particularly Tricomix, as a biostimulant alternative to improve the quality of banana seedlings under nursery conditions before field establishment.

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Compliance with ethical standards. This study did not involve human participants or animals. Therefore, approval by an ethics committee was not required.

Data availability. The raw data supporting the conclusions of this article will be made available by the authors on request.

Author contribution statement (CRediT). **J.A. Torres-Rodríguez –** Conceptualization, Methodology, Formal analysis and Writing-Review and editing. **M.R. Morejón-Centeno –** Visualization, Data Curation and Validation. **D. Castillo –** Supervision and Data Curation. **K. R. Mayorga-Morejon** – Supervision, Data Curation and Writing—review and editing., **E. A. Bustamante-Intriago** – Conceptualization, Funding acquisition and Project administration., **J. A. Alejandre Rosas –** Formal analysis, Software and Writing-Original Draft. **P. Preciado-Rangel –** Writing-Original Draft, Writing-Review and editing and Visualization.

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