Accepted manuscript

Cruz-Lara, D.A., E. Polanco-Díaz, Y.A. Deaquiz-Oyola, M. Betancourt-Vásquez, and G.A. Rodríguez-Yzquierdo. 2024. Evaluation of the effect of biostimulant substances on the growth and physiological response of Dominico-Hartón plantain (*Musa* AAB). Revista Colombiana de Ciencias Hortícolas 18(3), e17926. Doi: https://doi.org/10.17584/rcch.2024v18i3.17926

Evaluation of the effect of biostimulant substances on the growth and physiological response of Dominico-Hartón plantain (*Musa* AAB)[1]

Evaluación del efecto de sustancias bioestimulantes en el crecimiento y respuesta fisiológica de plátano Dominico-Hartón (Musa AAB)

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1This article is a product of the research group: "Grupo de Investigaciones Agrícolas – GIA" of the *Facultad Ciencias Agropecuarias* of the *Universidad Pedagógica y Tecnológica de Colombia* – UPTC.

Plantain Dominico-Hartón in nursery stage.
Photo: D.A. Cruz-Lara.

Short title: BIOSTIMULANT SUBSTANCES IN DOMINICO-HARTÓN PLANTAIN (*MUSA* AAB)

Doi: https://doi.org/10.17584/rcch.2024v18i3.17926

Received: 06-07-2024 Accepted: 17-09-2024 Published: 07-11-2024

ABSTRACT

The physiological quality of plantain plants (*Musa* AAB) is an important attribute to enhance multiplication efficiency and the adaptation of plant material to field conditions. The effect of applying biostimulants substances on the growth and physiological response of plantain vitroplants in the nursery was evaluated. The trial was conducted under a completely randomized block design in four municipalities of the Department of Huila, Colombia. The evaluated treatments were *Trichoderma koningiosis* (Tk), *Bacillus amyloliquefaciens* (Ba), Humic extract + Fulvic acids + free amino acids (Eh+Af+Al), Chitosan (Qt), *Bacillus subtilis* (Bs), Silicon dioxide (Ds), Salicylic acid (As), and Water Control (T). Plant height, pseudostem diameter, total leaves, functional leaves, dry biomass of leaves, pseudostem, corm, and root, as well as photosynthesis (A), stomatal conductance (Gs), transpiration (Tr), and chlorophyll index (CI) were evaluated at 90 days after planting. Improvements in the physiological response of treated plants compared to the control were evident; however, Tk significantly improved its physiological behavior in gas exchange, with increases in Gs, Tr, and CI. A differential effect of the biostimulants by locality in treated plants was observed, due to the significant increase in leaf dry matter with Qt, As, Eh+Af+Al, and Ds and in plant height with treatments Qt, As, and Tk. These results demonstrate the advantages of using biostimulants to promote the growth and physiological quality of plantain plants in nursery stage.

Additional key words: Musaceae; gas exchange; development; biomass; plant nurseries.

RESUMEN

La calidad fisiológica de las plantas de plátano (*Musa* AAB) es un atributo importante para mejorar la eficiencia de la multiplicación y adaptación del material vegetal a las condiciones de campo. Se evaluó el efecto de la aplicación de sustancias bioestimulantes sobre el crecimiento y la respuesta fisiológica de vitroplántulas de plátano en vivero. El ensayo se realizó bajo un diseño en bloques completamente al azar en 4 municipios del departamento del Huila, Colombia. Los tratamientos evaluados fueron *Trichoderma koningiosis* (Tk), *Bacillus amyloliquefaciens* (Ba), Extracto húmico+ Ácidos fúlvicos + aminoácidos libres (Eh+Af+Al), Quitosano (Qt), *Bacillus subtillis* (Bs), Dióxido de silicio (Ds), ácido salicílico (As) y Testigo agua (T). Se evaluó en la planta la altura, diámetro de pseudotallo, hojas totales, hojas funcionales, biomasa seca de hojas, pseudotallo, cormo y raíz, así como fotosíntesis (A), conductancia estomática, (Gs), transpiración (Tr) e índice de clorofila (IC) a los 90 días después de la siembra. Se evidenció mejoras en respuesta fisiológica de plantas tratadas comparado al testigo, sin embargo, Tk mejoró significativamente su comportamiento fisiológico en el intercambio de gases, con incremento en Gs, Tr e IC. Se observó un efecto diferencial de los bioestimulantes por localidad en plantas tratadas, debido al incremento significativo en la materia seca en hoja con Qt, As, Eh+Af+Al y Ds y en altura de planta con los tratamientos Qt, As y Tk. Estos resultados demuestran las ventajas del uso de bioestimulantes sobre la promoción de crecimiento y calidad fisiológica de plantas de plátano en etapa de vivero.

Palabras clave adicionales: Musaceae; intercambio de gases; desarrollo; biomasa; plantas de vivero.

INTRODUCTION

Plantain (*Musa* AAB) is a food resource that plays an important socioeconomic role for developing countries in tropical and subtropical areas (Loranger-Merciris *et al*., 2023). In Colombia, it represents the fruit with the largest planting area, with about 493,000 ha, a production of 4,370,751 t, and a yield of 7.7 t ha^{-1} In the Department of Huila, the municipalities of Timana, Garzon, Palermo, and Santa Maria are notable for plantain production, contributing 28% of the department's total output (UPRA, 2023). The Dominico-Hartón clone is producing regions between 1,000 to 1,800 m.a.s.l., either as monoculture or in association with other crops such as coffee, cocoa, and agroforestry systems (Polanco *et al*., 2024).

Plantain, like other edible Musaceae, requires solutions to the limited availability of quality planting material and low uniformity in field plant establishment, which can be ensured with plant material propagated in nurseries, with proper management and protection against pests and pathogens (Ugarte-Barco *et al*., 2022). Regarding plantain seed production, it traditionally does not involve significant technological developments; however, many alternatives have been validated by research that improve the efficiency of material production, as well as its quality attributes (Polanco *et al*., 2024).

The use of plant bioestimulants has garnered significant attention in sustainable agricultural production from an environmental perspective, as it offers the possibility of reducing the chemical footprint of agricultural products. When applied to plants or soil, they can increase nutrient absorption and distribution (Mandal *et al*., 2023), can be used as a complement to fertilizers and phytosanitary products, with the potential to enhance tolerance to water stress, salinity, temperature, and changes related to the oxidation-reduction reaction (Nephali *et al*., 2020). These products include beneficial bacteria and fungi, peptides, phenolic compounds, hormones, saccharides, free amino acids, protein hydrolysate derivatives, seaweed (Distefano *et al*., 2022).

Biostimulants products have reported uses and beneficial effects in various crops, notably chitosan (Chakraborty *et al*., 2020), silicon (Bishnoi *et al*., 2023), and microbial origins such as *Trichoderma* (Khalil and Youssef, 2024) and *Bacillus* (Dame *et al*., 2021). The benefits that biostimulants offer in promoting growth and seed health have been studied in other plantain clones (Mateus-Cagua *et al.,* 2024). However, the diverse environmental conditions present in the production areas of the Dominico-Hartón clone in the department of Huila allow for differential expression in plant development, and thus, the interaction between the biostimulant substance and the plant may yield different responses.

The production of seed in the nursery stage relies on the use of asexual seed (corms) (Wagner-Medina *et al*., 2023); however, this type of material is heterogeneous in initial vegetative development and carries a high risk of contamination by pathogens such as *Fusarium sp.* and *Ralstonia solanacearum* Race 2. Therefore, the use of meristematic tissue culture, sourced from certified laboratories, mitigates these risks (Betancourt *et al*., 2021). The objective of the present work was to necessitating a deeper evaluation of different biostimulants substances with potential for cultivation, specifically with the Dominico-Hartón clone, in nursery stage, and their effect on physiological processes and growth of vitroplants.

MATERIALS AND METHODS

Locality and experimental setup

The study was conducted in four representative municipalities of plantain production in the Department of Huila, Colombia (Santa Maria, Palermo, Timana, and Garzon). These localities represent the agroecological regions (bh-PM) Premontane Rainforest (Hilje and Jiménez-Saa, 2017) with average temperatures ranging from 17°C to 27.1°C and average monthly rainfall between 80.7 and 153 mm (Tab.1) (Ideam, 2024 and Fedearroz, 2024). The plant material used was Dominico-Hartón plantain vitroplants, hardened with four functional leaves at the time of planting. One plant was sown per 2 kg capacity bag with a substrate based on black soil, burned rice husk, and compost in proportions 1:2:1. The substrate characteristics were as follows: pH of 5.1, effective cation exchange capacity of 12.2 $cmol_{(+)}$ kg^{-1}, and electrical conductivity of 0.66 dS m^{-1}. Other chemical characteristics: organic nitrogen 0.1%, organic matter 2.5%, Ca 30.9 $cmol_{(+)}$ kg^{-1}, K 0.7 $cmol_{(+)}$ kg^{-1}, Mg 1.0 $cmol_{(+)}$ kg^{-1}, P 550 mg kg^{-1}, Cu 0.23 mg kg^{-1}, Fe 83.72 mg kg^{-1}, Mn 1.93 mg kg^{-1}, Zn 2.07 mg kg^{-1}, and B 0.82 mg kg^{-1}. Sandy loam texture with 2.90% clay, 41.45% silt, and 55.65% sand. The plants underwent an acclimatization stage for 1 month in the nursery with a shade cover allowing 70% light entry. The trial was carried out from November 2022 to January 2023.

Table 1. Geographical location and climatic conditions of the four localities.

Locality	Latitude	Longitude	Altitude (m.a.s.l)	Average temperature (°C)	Average solar brightness (h d^{-1})	Average monthly precipi-tation (mm)
Santa Maria	2°55'36"N	75°35'56"W	1,450	17.00	4.51	153.00
Palermo	2°48'18"N	75°30'10"W	1,132	27.12	4.55	127.00
Timana	1°57'02"N	75°55'48"W	1,131	21.54	4.04	80.73
Garzon	2°15'26"N	75°30'21"W	1,417	19.90	3.13	89.13

Source: Ideam (2024) and Fedearroz (2024).

The substrate was irrigated at 80% of field capacity, using a sprinkler irrigation system. Seedling fertilization was done foliar every 7 d based on macro and microelements at a dose of 3 mL L^{-1} of water (Formador 2000®, Halcon Agroindustrial, Cajica, Colombia) and soil fertilization at 40 days after transplanting (dat), applying to each plant (g): 2 urea (Nutrimon®), 2 ammonium phosphate (Nutrimon®), 2 potassium chloride (Nutrimon®, Monomeros Colombo Venezolanos, Barranquilla Colombia), and 1 Borozinco® (Microfertiza, Bogota, Colombia).

Growth and development

Growth variables were measured at 90 dat, four plants per experimental unit (EU) were evaluated for plant height (cm), measured from the substrate level to the insertion on the first leaf from apex to base. Pseudostem diameter (mm) was measured 5 cm above the substrate level with a Mitutoyo digital caliper. Two plants per EU were taken, separated by organs (leaves, pseudostem, corm, and root), and weighed fresh with a BBG precision scale (model LAB 3000), also, dry matter accumulation (DM) by organ was evaluated (60 d after applying the treatments) with samples of each organ dried in an oven (Memmert Model 600, Schwabach, Germany) at 65°C for 72 h. The number of total and functional leaves emitted was recorded every 15 d (Mateus-Cagua *et al*., 2024).

Chlorophyll index measurement (CI): It was measured with a chlorophyll meter (SPAD 502, Konica Minolta, Tokyo, Japan) in the middle third of the third leaf from apex to base (recently mature leaf), averaging four readings per leaf. Measurements of the mentioned variables were performed on two plants per experimental unit at 90 dat (Mateus-Cagua *et al*., 2024).

Photosynthesis and gas exchange: A portable infrared gas analyzer, model LI-6400XT (LICOR, Inc., Lincoln, Nebraska), equipped with a red-blue LED artificial chamber (6400-02B) was used. Evaluations were conducted between 9:00 and 11:30 am, with the photosynthetic photon flux density adjusted to 300 µmol m^{-2} s^{-1} prior to a light saturation curve while the CO_2 concentration was set to 400 µmol m^{-2} s^{-1}; data were recorded when the coefficient of variation was less than 5%. The variables evaluated were net photosynthesis rate (A), transpiration rate (Tr), stomatal conductance (Gs) (Mateus-Cagua *et al*., 2024). This measurement was performed at 90 dat.

Experimental design

Four experiments were established, one per locality. Eight treatments were evaluated, with 4 repetitions and 4 plants per experimental unit, 8 treatments distributed in a completely randomized block design. The treatments were *Trichoderma koningiosis* (Tk), concentration 1×10^9 conidia/g (1 g L^{-1}); *Bacillus amyloliquefaciens* (Ba) concentration $1x10^9$ CFU/mL, 25 mL L^{-1}; humic extract + fulvic acids + free amino acids (Eh+Af+Al), 3 mL L^{-1}; chitosan (Qt), 3 mL L^{-1}; *Bacillus subtilis* (Bs) concentration 5.13×10^{10} UFC/mL, 10 mL L^{-1}; silicon dioxide (Ds), 4 g L^{-1}; salicylic acid (As), 8 mL L^{-1} and control (T) without product application (tap water). The treatments were administered by drench at 25 cc solution per plant, starting 30 dat in the bags and continuing until day 90, with applications every 2 weeks.

Table 2. Commercial information on products used in treatments.

Treatment	Description	Manufacturer	Production city
Tk	Tricotec®	Agrosavia	Mosquera, Colombia
Ba	Natibac®	Agrosavia	Mosquera, Colombia
Eh+Af+Al	Agriful®+ Tecamin®	Agrisec	Quito, Ecuador
Qt	KaitoSol®	Advanced green nanote-chnologies	Putrajaya, Malasia
Bs	Serenade®	Bayer	Tlaxcala, Mexico
Ds	Tierra de diatomeas®	Agropuli	Bogota, Colombia

As	ReLeaf®	Stoller	Zipaquira, Colombia

Statistical analysis

The data were first analyzed for each locality for the growth and dry matter accumulation variables, followed by a joint analysis of the four localities for physiological variables. Tests for normality, homogeneity of variance, and independence assumptions were performed, followed by Analysis of variance to determine statistically significant differences between treatments and multiple comparison tests by Tukey, with a significance level of 0.05. A heat map was created to understand the relationship between the evaluated variables in the four localities, analyzed with Pearson's correlation coefficient and principal component analysis with the data set from the 4 locations. Data were analyzed using R Studio software version 3.3.0.

RESULTS AND DISCUSSION

Growth parameters

The biostimulant treatments that showed significant differences ($P\leq0.05$) with improved plant height response varied by locality. In Santa Maria, Qt increased plant height by 20.8% compared to the control (water), in Palermo, As by 14.9%, in Timana, Tk and Ba treatments showed increases of 14.43 and 13.75%, respectively, and in Garzon, Tk by 26.8%, As by 23.5%, Ba by 22.08%, Ds by 21.18%, and Qt by 19.14% (Tab. 3).

Table 3. Influence of biostimulants on growth and development variables in 'Dominico-Hartón' plantain plants at 90 days.

Locality	Treatment	Height (cm)	Pseudostem diameter (mm)	Functional leaves	Total leaves
Santa Maria	Tk	20.76±4.25 b	17.63±3.04	7.15±0.88	8.28±1.02
	Ba	22.69±4.20 ab	19.46±2.36	6.29±0.81	8.08±0.84
	Eh+Af+Al	22.42±3.86 ab	18.85±3.22	6.73±1.09	8.04±1.07
	Qt	25.31±2.88 a	19.08±2.52	6.30±1.03	8.04±0.48
	Bs	23.58±2.87 ab	18.87±2.04	6.34±1.17	8.03±1.05
	Ds	23.71±3.29 ab	18.14±2.58	6.16±0.79	7.78±0.85
	As	22.86±3.05 ab	20.56±2.27	5.96±0.57	7.97±0.97
	T	20.95±3.07 ab	19.70±2.19	6.13±1.05	8.01±0.93

	P-value	0.025371*	0.19354	0.07051	0.9596
Palermo	Tk	27.40±3.02 ab	22.02±2.18 b	5.1±1.10 ab	5.06±0.99 b
	Ba	25.24±4.58 b	23.13±2.37 ab	5.2±1.68 ab	5.36±1.24 b
	Eh+Af+Al	27.70±3.27 ab	23.68±2.65 ab	5.5±1.17 ab	5.32±1.41 b
	Qt	26.67±2.00 ab	23.53±2.30 ab	6.4±1.07 a	6.78±0.78 a
	Bs	24.77±2.38 b	22.90±2.49 ab	5.2±1.61 ab	5.34±0.91 b
	Ds	25.41±2.36 b	22.80±2.57 ab	5.1±1.10 ab	5.24±0.72 b
	As	30.02±1.87 a	25.58±2.59 a	4.1±0.99 b	4.56±0.70 b
	T	26.12±1.73 b	22.52±1.65 ab	4.6±1.07 b	4.94±0.93 b
	P-value	0.001371 **	0.05126	0.01381*	0.0007243***
Garzon	Tk	29.76±3.54 a	23.95±3.28	7.55±0.76	7.94±0.70
	Ba	28.64±3.80 a	23.16±3.25	6.80±0.63	7.02±0.70
	Eh+Af+Al	26.88±3.29 ab	23.18±2.66	6.40±1.07	6.76±1.14
	Qt	27.95±3.16 a	23.00±2.51	7.00±0.94	7.42±1.00
	Bs	26.14±4.69 ab	21.76±3.28	7.10±0.99	7.60±0.97
	Ds	28.43±3.07 a	23.67±3.47	6.90±0.73	7.38±0.72
	As	28.98±2.14 a	23.15±2.56	7.00±0.66	7.22±0.76
	T	23.46±1.83 b	21.14±1.77	7.30±1.05	7.44±1.04
	P-value	0.0001151 ***	0.33943	0.1762	0.1481
Timana	Tk	31.87±3.60 a	22.47±2.99	7.6±0.69	8.04±0.75
	Ba	31.68±3.18 a	22.58±2.55	7.2±0.78	7.56±0.82
	Eh+Af+Al	27.90±4.24 b	20.45±3.54	7.1±0.99	7.54±0.83
	Qt	29.94±2.88 ab	22.55±2.85	7.1±0.99	7.60±0.88
	Bs	30.54±4.56 ab	22.31±2.47	7.4±0.51	7.80±0.87
	Ds	30.53±3.89 ab	21.98±2.28	7.8±0.63	8.24±0.66
	As	31.16±4.44 ab	22.83±2.67	7.0±1.24	7.44±1.26
	T	27.85±1.58 b	20.86±1.35	7.6±0.69	8.00±0.84
	P-value	0.04846*	0.3512	0.3454	0.4062

Means within columns with different letters differ statistically according to Tukey's test ($P \leq 0.05$) (n=10). Tk: *T. koningiosis*, Ba: *B. amyloliquefaciens*, Eh+Af+Al: humic extract + fulvic acids + free amino acids, Qt: chitosan, Bs: *B. subtilis*, Ds: silicon dioxide, As: salicylic acid, and T: control

Growth response to the microorganism-based biostimulants (Tk and Ba) was observed in two localities, Garzon and Timana, which had intermediate environmental conditions between Palermo and Santa Maria, with higher rainfall and contrasting temperatures (27.12 - 17°C). According to Etesami and Glick (2024), the response of treated plants may be influenced by locality. *Trichoderma* promotes growth by producing vitamins, solubilizing nutrients, synthesizing phytohormones like auxins and cytokinins, and enhancing root development (Rodríguez-García and Vargas-Rojas, 2022; Mandal *et al*., 2023). *B. amyloliquefaciens* treatment showed significant growth promotion, attributed to bacterial synthesis of IAA, which responds to environmental signals and induces changes in root architecture and nutrient uptake (Keswani *et al*., 2020).

In Palermo, As showed a growth-promoting effect on plant height and pseudostem diameter, with significant differences ($P \leq 0.05$) (Tab. 3) and a 13.58% increase compared to the control treatment in pseudostem diameter. As plays a role in growth and development under both stress and non-stress conditions (Khan *et al*., 2020) and influences many physiological and metabolic processes, strengthening the oxidative defense system (Kaya *et al*., 2023).

In Santa Maria and Garzon, the localities with the lowest temperatures in the study (17°C and 19.9°C), the Qt treatment resulted in greater plant height. This growth stimulation is attributed to increased water and mineral absorption, nitrogen input to the soil derived from chitin, and phytohormone synthesis (Chakraborty *et al*., 2020; Stasińska-Jakubas and Hawrylak-Nowak, 2022). The environmental conditions in Garzon allowed the Tk and Ba (microbial origin) and As and Ds (non-microbial) treatments to significantly increase plant height. According to Ramírez-Olvera *et al*. (2021) and Raza *et al*. (2023), Ds stimulates plant growth in terms of height, leaf number, and biomass by modulating the concentration of vital biomolecules and nutrients, in addition to reducing water loss from the cell wall.

The total number of leaves was between 7-8 leaves and functional leaves between 6 and 7 at the measurement made at 90 d in the localities of Garzon, Timana, and Santa Maria, indicating good agronomic management during the evaluation period since no leaves were removed due to sanitary problems; however, in the locality of Palermo, this value ranged between 4 and 6 in total and functional leaves, due to the removal of senescent leaves at 75 d. In this last locality, significant differences ($P \leq 0.05$) were found between treatments, with the highest total and functional leaf emission rate observed in plants treated with Qt, with an increase of 37.2% and 39.1% compared to the control for total and functional leaves.

Dry matter accumulation (DM)

Dry matter accumulation per plant was highest in Palermo, ranging from 18 to 25 g per plant across the treatments evaluated, while in Santa Maria, Garzon, and Timana, values ranged from 9 to 16 g per plant. Palermo had the highest temperature and precipitation among the four localities. According to the obtained results (Fig. 1), significant differences ($P \leq 0.05$) were found between treatments for the different tissues evaluated across the four localities. Compared to the control, the As-based treatment showed an increase in leaf dry matter of 73.7% and 50.2% in Santa Maria and Garzon, which are the cooler localities. Similarly, treatments with Qt and Eh+Af+Al showed a positive effect, with leaf dry matter gains of 70.6% and 73.0% in Santa Maria and 16.5% and 15.1% in Timana, respectively. In Palermo, the Ds treatment achieved the highest increase (40.1%).

In turn, DM accumulation in the pseudostem showed significant differences in the localities of Santa Maria and Garzon, as did leaf DM. Treatments based on Eh+Af+Al, Qt, and As showed increases of 77.2%, 71%, and 64.3% in DM compared to the control in Santa Maria, and in Garzon, Ba increased DM by 56.8%. Dry matter accumulation in the root also showed significant differences between treatments, with Eh+Af+Al and As increasing DM by 73% and 40.7% compared to the control in Santa Maria. In Garzon, the Tk treatment and in Timana, Qt showed increases of 55.5% and 23.5%, respectively.

The effect of biostimulant substances on growth promotion across plant organs has been studied in a wide variety of crops (Rana *et al*., 2022). According to Mateus-Cagua and Rodríguez-Yzquierdo (2019), the distribution of DM among plant organs showed that the highest percentage accumulated during the vegetative phase was in the leaf, followed by the pseudostem, corm, and root. The Eh+Af+Al treatment showed significant differences in leaf, pseudostem, and root. With the use of humic biostimulants, beneficial effects have been observed in increasing plant height and root weight in bananas and plantains (Martínez *et al*., 2021). Moreover, Mateus-Cagua and Rodríguez-Yzquierdo (2019) demonstrated changes in root length and architecture, primarily in adventitious and lateral roots. Humic extracts (Eh) and fulvic acids (Af) are compounds that enhance soil fertility, root nutrition, and soil cation exchange capacity (Dubey

Accepted manuscript

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and Sharma, 2023). Free amino acids (Al) are protein hydrolysates containing nitrogen that boost basic nutrition and plant growth (Vasconcelos and Chaves, 2020).

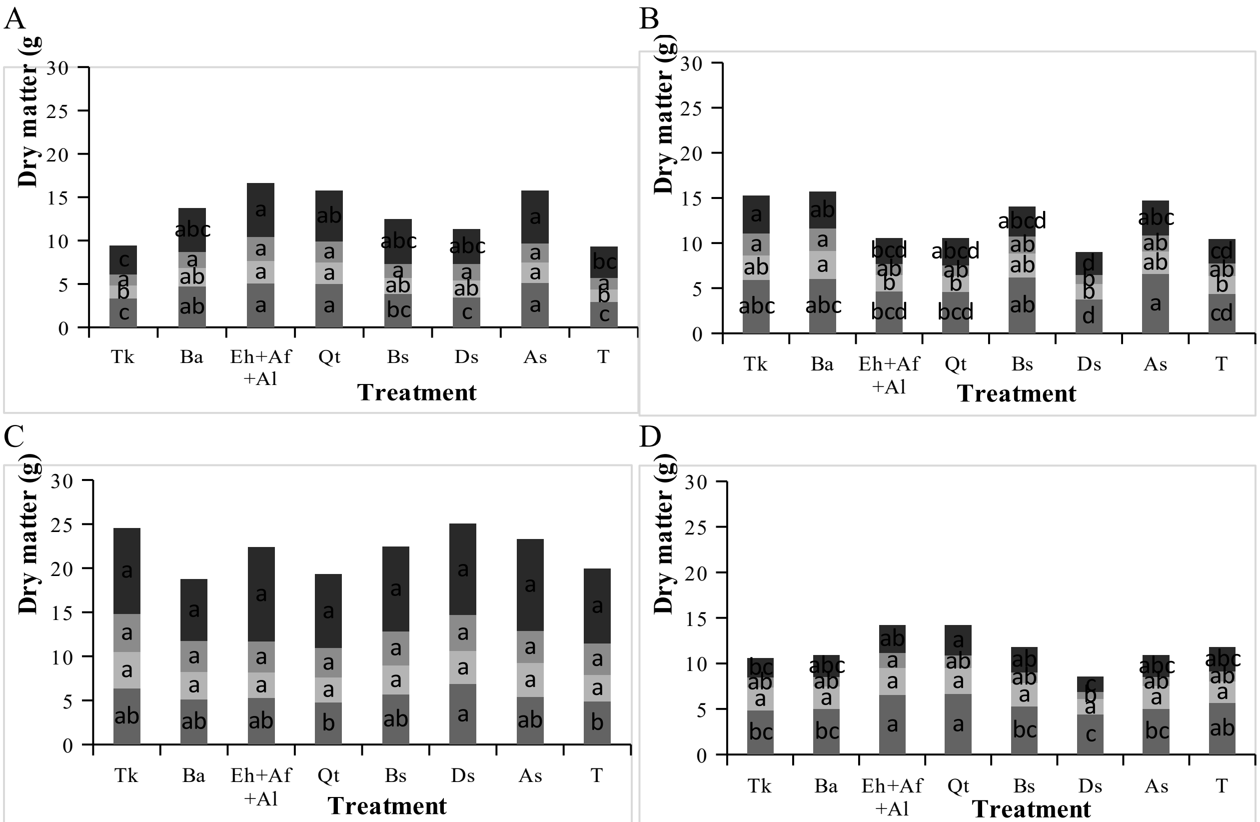

Figure 1. Dry matter in plants by organ. Locality: A. Santa Maria; B. Garzon; C. Palermo; D. Timana. Means (*n*=8) within columns with different letters differ statistically according to Tukey's test ($P\leq0.05$). Tk: *T. koningiosis*, Ba: *B. amyloliquefaciens*, Eh+Af+Al: humic extract + fulvic acids + free amino acids, Qt: chitosan, Bs: *B. subtilis*, Ds: silicon dioxide, As: salicylic acid, and T: control.

Photosynthetic activity

Results by locality did not show significant differences between treatments except for transpiration in Santa Maria and photosynthesis in Palermo (data not shown). However, statistically significant differences ($P\leq0.05$) were found between the treatment with application of biostimulant substances and the variables stomatal conductance (Gs), transpiration (Tr) for the entire pool of data for each treatment from the four localities evaluated (Fig. 2).

Plants treated with biostimulants showed higher values compared to the control treatment. Tk showed significant differences ($P\leq0.05$) among treatments, with an increase of 62.2% in Gs and 48.9% in Tr. In photosynthesis (A) and chlorophyll index (CI), no significant differences were

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found between treatments ($P \leq 0.05$). However, all biostimulant treatments showed a higher photosynthetic rate (A) than the control.

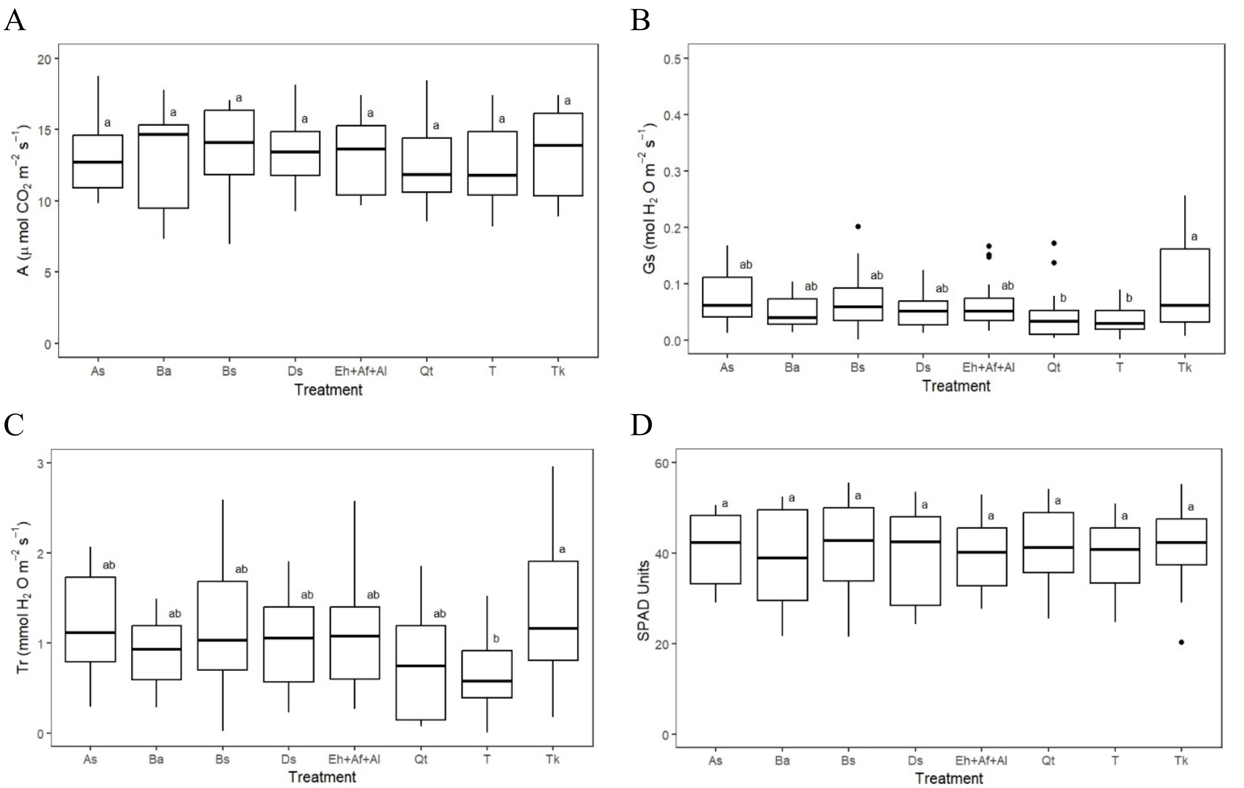

Figure 2. Physiological behavior variables in plants with the application of biostimulant substances in four localities. A: photosynthesis (A), B: stomatal conductance (Gs), C: transpiration (Tr), D: chlorophyll index (Spad). Tk: *T. koningiosis*, Ba: *B. amyloliquefaciens*, Eh+Af+Al: humic extract + fulvic acids + free amino acids, Qt: chitosan, Bs: *B. subtilis*, Ds: silicon dioxide, As: salicylic acid, and T: control. Means (*n*=16) within columns with different letters differ statistically according to Tukey's test $P \leq 0.05$.

CI followed a similar trend to Gs and Tr, except for the Ba treatment (38.53), which had a lower performance than the control (39.16 SPAD units). In Musaceae, normal CI values range from 40 to 50, and values below 30 indicate a stress condition that impairs physiological function in the plant (Mateus-Cagua *et al*., 2024). In this context, the values observed align with the optimal range for this variable. According to results obtained by Yasmeen and Siddiqui (2017) and Khalil and Youssef (2024), the application of *Trichoderma harzianum* enhances photosynthetic pigments, positively modulates physiological and biochemical processes, and increases stomatal conductance and photosynthetic efficiency in other crops.

Multivariate analysis

Correlation coefficient

Figure 3 shows a very strong positive correlation ($P \leq 0.001$) between stomatal conductance (Gs) and transpiration (Tr), total and functional leaves (0.90); fresh root with dry corm weight, fresh and dry leaf weight (0.85); fresh leaf weight and fresh pseudostem weight (0.81); and fresh corm weight with dry pseudostem weight (0.81). The positive relationship between Gs and Tr (Fig. 3 and 4) indicates the physiological state, health of a plant, and how it is affected by experimental conditions, making them good predictors of crop development and productivity (Ospina-Salazar *et al*., 2018). This correlation has been studied in various crops. In sugarcane (*Saccharum* spp.), high and significant correlations were observed between stomatal conductance, transpiration rate, and chlorophyll index (CI) before harvest (Mendez-Adorno *et al*., 2016).

A considerable negative correlation was also found between growth and biomass variables (total and functional leaves, pseudostem perimeter, and fresh and dry corm and root weight) with the chlorophyll index (CI) (-0.52 to -0.75). (Fig. 3 and 4). This may be attributed to the high biomass formation of different organs at 90 d, but it is more negative in root biomass (-0.76), which creates a restricted condition in root distribution in the substrate, reducing the formation of photosynthetic pigments and consequently lowering the chlorophyll index in the plant. According to Rizzardi *et al.* (2001), competition among plant roots interferes with water and nutrient availability, occurring when the depletion zone exceeds itself.

In contrast, no relationship was found between photosynthesis and growth and biomass accumulation variables ($P > 0.05$), presenting a weak positive correlation for plant height (0.05), dry and fresh leaf weight (0.13 and 0.06), and fresh and dry pseudostem weight (0.02). A weak negative correlation was found between total and functional leaves, pseudostem perimeter, and fresh and dry corm and root weight (-0.01 to -0.09).

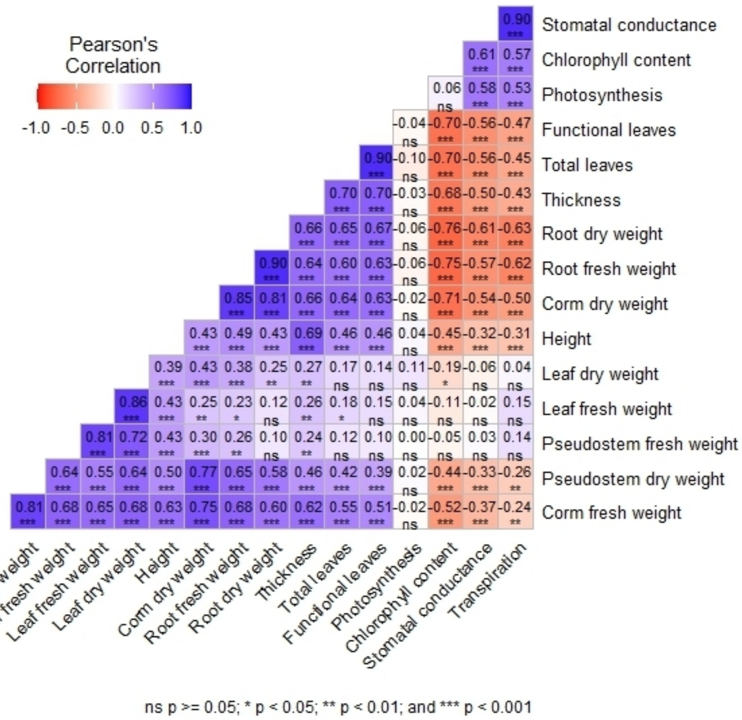

Figure 3. Pearson correlation coefficient for growth and physiological behavior variables in plantain cv. Dominico-Hartón.

Principal component analysis

The analysis incorporated the data of the evaluated variables in the two main dimensions PC1=49% and PC2=20%, representing 69% of the data variability (Fig. 4). In general, the evaluated physiological variables (A, Tr, Gs, and CI) were distributed in the right quadrant of the PC1 scatter plot, and variables associated with growth and biomass accumulation were in the left quadrant of PC2. Figure 4 shows cluster formation by locality. Timana, Santa Maria, and Garzon have greater expression in physiological parameters such as photosynthesis (A), transpiration (Tr), stomatal conductance (Gs), and chlorophyll index (CI), in contrast to Palermo, which showed a negative correlation in these parameters but obtained a greater expression of biomass variables in root, corm, and leaves. Palermo experienced warmer conditions with higher temperature and solar radiation compared to the other three localities. According to Restrepo-Díaz and Sánchez-Reinoso (2020), temperature (high or low) alters source-sink relationships as it can reduce the supply of photoassimilates from the source, which is related to plant photosynthesis and the solar radiation intercepted by the plant, affecting biomass accumulation in the plant.

For this study, local climatic conditions influenced the expression of variables associated with physiological behavior, growth, and biomass production. The variables that determine the effect of the treatments are related to biomass accumulation, such as dry matter in different organs, correlated with growth parameters such as pseudostem diameter, plant height, leaf emission, stomatal conductance, transpiration, and chlorophyll index.

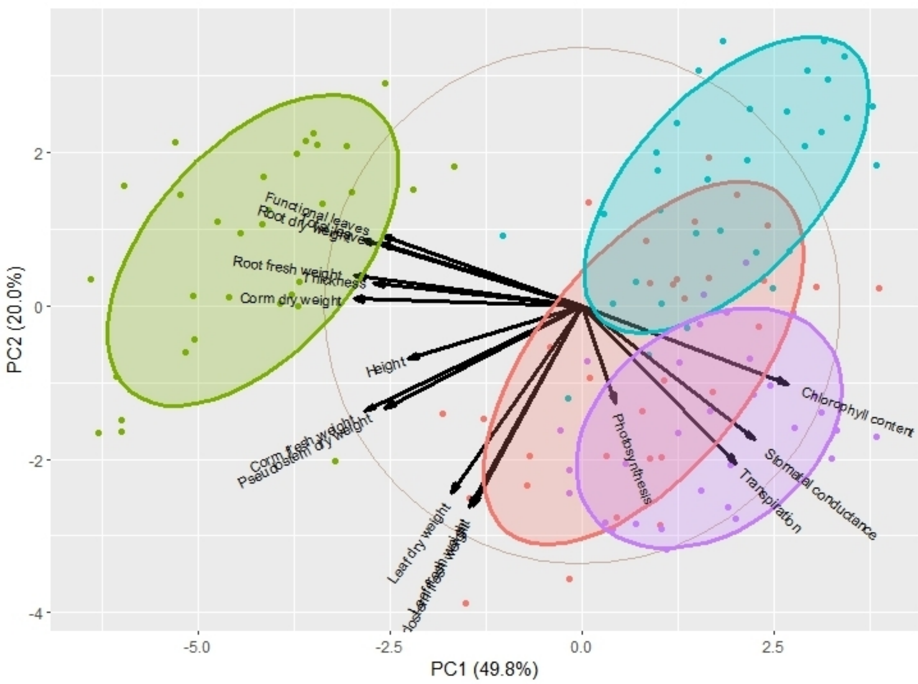

Figure 4. Biplot of data using principal component analysis of growth and physiological behavior variables.

CONCLUSION

There was an increase in growth variables, height, and pseudostem diameter in plants treated with Chitosan (Qt), *Trichoderma koningiosis* (Tk), Salicylic Acid (As), *Bacillus amyloliquefaciens* (Ba), and Silicon Dioxide (Ds) treatments. The biostimulants Salicylic Acid (As), Chitosan (Qt), and the combination of Humic Extract, Fulvic Acids, free amino acids (Eh+Af+Al), and *Bacillus amyloliquefaciens* (Ba) presented a positive effect on biomass accumulation in leaf, stem, and root.

The use of biostimulants substances in plantain at the nursery stage significantly influences physiological behavior and gas exchange parameters such as transpiration and stomatal conductance. The performance of treatments is influenced by local environmental conditions;

therefore, the recommendation for the use of biostimulants should be differentiated based on a specific environment or locality.

Acknowledgments

To the Pedagogical and Technological University of Colombia, Faculty of Agricultural Sciences, and Colombian Corporation for Agricultural Research (Agrosavia), for the accompaniment in the research process.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

Betancourt, M., J. Cárdenas, and G.A. Rodríguez. 2021. Guía para importar a Colombia germoplasma y material de propagación de plátano y banano en el marco de la emergencia sanitaria por *Foc* R4T en Colombia. Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Mosquera, Colombia. Doi: https://doi.org/10.21930/agrosavia.manual.7404753

Bishnoi, A., P. Jangir, P.K. Shekhawat, P.K., Ram, H., and P. Soni. 2023. Silicon supplementation as a promising approach to induce thermotolerance in plants: current understanding and future perspectives. J. Soil Sci. Plant Nutr. 23, 34-55. Doi: https://doi.org/10.1007/s42729-022-00914-9

Chakraborty, M., M. Hasanuzzaman, M. Rahman, M.A.R. Khan, P. Bhowmik, N.U. Mahmud, M. Tanveer, and T. Islam. 2020. Mechanism of plant growth promotion and disease suppression by chitosan biopolymer. Agriculture 10(12), 624. Doi: https://doi.org/10.3390/agriculture10120624

Dame, Z.T., M. Rahman, and T. Islam. 2021. Bacilli as sources of agrobiotechnology: recent advances and future directions. Green Chem. Lett. Rev. 14(2), 246-271. Doi: https://doi.org/10.1080/17518253.2021.1905080

Distefano, M., C.B. Steingass, C. Leonardi, F. Giuffrida, R. Schweiggert, and R.P. Mauro. 2022. Effects of a plant-derived biostimulant application on quality and functional traits of greenhouse cherry tomato cultivars. Food Res. Int. 157, 111218. Doi: https://doi.org/10.1016/j.foodres.2022.111218

Dubey, S.C. and K. Sharma. 2023 Biostimulant: an innovative approach for sustainable crop production. Current Sci. 125(4), 377-382.

Etesami, H. and B.R. Glick. 2024. Bacterial indole-3-acetic acid: a key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. Microbiol. Res. 281, 127602. Doi: https://doi.org/10.1016/j.micres.2024.127602

Fedearroz, Federación Nacional de Arroceros Colombia. 2024. Portal agroclimático. In: https://clima.fedearroz.com.co/; consulted: January, 2024.

Hilje, L. and H. Jiménez-Saa. 2017. Leslie R. Holdridge: un botánico que vio muy lejos. Rev. Cienc. Ambient. 51(2), 181-194. Doi: https://doi.org/10.15359/rca.51-2.10

Ideam, Instituto de Hidrología, Meteorología y Estudios Ambientales Colombia. 2024. Sistema de Información para la gestión de datos Hidrológicos y Meteorológicos – DHIME: datos hidrológicos y meteorológicos. In: http://www.dhime.ideam.gov.co/webgis/home/; consulted: January, 2024.

Kaya, C., F. Ugurlar, M. Ashraf, and P. Ahmad. 2023. Salicylic acid interacts with other plant growth regulators and signal molecules in response to stressful environments in plants. Plant Physiol. Biochem. 196, 431-443. Doi: https://doi.org/10.1016/j.plaphy.2023.02.006

Khalil, M.I.I. and S.A. Youssef. 2024. Physiological and biochemical responses of *Alternaria alternata* infected tomato to *Trichoderma harzianum* and *Chaetomium globosum* application. S. Afr. J. Bot. 166, 116-125. Doi: https://doi.org/10.1016/j.sajb.2024.01.020

Khan, N., A. Bano, S. Ali, and M.A. Babar. 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Reg. 90(2), 189-203. Doi: https://doi.org/10.1007/s10725-020-00571-x

Keswani, C., S.P. Singh, L. Cueto, C. García-Estrada, S. Mezaache-Aichour, T.R. Glare, R. Borriss, S.P. Singh, M.A. Blázquez, and E. Sansinenea. 2020. Auxins of microbial origin and their use in agricultura. Appl. Microbiol. Biotechnol. 104, 8549-8565. Doi: https://doi.org/10.1007/s00253-020-10890-8

Loranger-Merciris, G., G. Damour, B. Deloné-Louis Jeune, H. Ozier-Lafontaine, M. Dorel, J. Sierra, J.-L. Diman, and P. Lavelle. 2023. Management practices and incidence of pests in plantain (*Musa paradisiaca* AAB) crops. Consequences on the sustainability of the cropping systems. Appl. Soil Ecol. 189, 104904. Doi: https://doi.org/10.1016/j.apsoil.2023.104904

Mandal, S., U. Anand, J. López-Bucio, Radha, M. Kumar, M.K. Lal, R.K. Tiwari, and A. Dey. 2023. Biostimulants and environmental stress mitigation in crops: a novel and emerging approach for agricultural sustainability under climate change. Environ. Res. 233, 116357. Doi: https://doi.org/10.1016/j.envres.2023.116357

Martínez, G., J.C. Rey, R. Pargas, C. Guerra, E. Manzanilla, and H. Ramírez. 2021. Efecto de sustratos y fuentes orgánicas en la propagación de banano y plátano. Agron. Mesoam. 32(3), 808-822. Doi: https://doi.org/10.15517/am.v32i3.42490

Mateus-Cagua, D.M., A. González-Almario, M. Betancourt-Vasquez, and G.A. Rodriguez-Izquierdo. 2024. Physiological response induced by biostimulants on plantain plants (*Musa* AAB) under *Ralstonia solanacearum* race 2 stress. Rev. Ceres 71, e71019. Doi: https://doi.org/10.1590/0034-737x2024710019

Mateus-Cagua, D. and G. Rodríguez-Yzquierdo. 2019. Effect of biostimulants on dry matter accumulation and gas exchange in plantain plants (*Musa* AAB). Rev. Colomb. Cienc. Hortic. 13(2), 151-160. Doi: https://doi.org/10.17584/rcch.2019v13i2.8460

Mendez-Adorno, J.M., S. Salgado-García, L.C. Lagunes-Espinoza, J.R.H. Mendoza-Hernández, M. Castelán-Estrada, S. Cordova-Sanchez, and C.I. Hidalgo-Moreno. 2016. Relación entre parámetros fisiológicos en caña de azúcar (*Saccharum* spp.) bajo suspensión de riego previo a la cosecha. Agroproductividad 9(3), 15-20.

Nephali, L., L.A. Piater, I.A. Dubery, V. Patterson, J. Huyser, K. Burgess, and F. Tugizimana. 2020. Biostimulants for plant growth and mitigation of abiotic stresses: a metabolomics perspective. Metabolites 10(12), 505. Doi: https://doi.org/10.3390/metabo10120505

Ospina-Salazar, D.I., J.A. Benavides-Bolaños, O. Zúñiga-Escobar, and C.G. Muñoz-Perea. 2018. Photosynthesis and biomass yield in Tabasco pepper, radish and maize subjected to magnetically treated water. Corpoica Cienc. Tecnol. Agropecuaria 19(2), 307-321. Doi: https://doi.org/10.21930/rcta.vol19_num2_art:537

Polanco, E., D.A. Cruz, J.E. Muñoz, M. Betancourt, and G.A. Rodríguez. 2024. Producción de semilla de plátano de calidad Dominico-Hartón en el departamento del Huila. Corporación Colombiana de Investigación Agropecuaria – Agrosavia, Mosquera, Colombia. Doi: https://doi.org/10.21930/agrosavia.manual.7407129

Rana, V.S., S. Sharma, N. Rana, and U. Sharma. 2022. Sustainable production through biostimulants under fruit orchards. CABI Agric. Biosci. 3, 38. Doi: https://doi.org/10.1186/s43170-022-00102-w

Ramírez-Olvera, S.M., L.I. Trejo-Téllez, F.C. Gómez-Merino, L.M. Ruíz-Posadas, E.G. Alcántar-González, and C. Saucedo-Veloz. 2021. Silicon stimulates plant growth and metabolism in rice plants under conventional and osmotic stress conditions. Plants 10(4), 777. Doi: https://doi.org/10.3390/plants10040777

Raza, T., M. Abbas, Amna S. Imran, M.Y. Khan, A. Rebi, Z. Rafie-Rad, and N.S. Eash. 2023. Impact of silicon on plant nutrition and significance of silicon mobilizing bacteria in agronomic practices. Silicon 15, 3797-3817. Doi: https://doi.org/10.1007/s12633-023-02302-z

Restrepo-Díaz, H. and A.D. Sánchez-Reinoso. 2020. Ecophysiology of fruit crops: a glance at its impact on fruit crop productivity. pp. 59-66. In: Srivastava, A.K. and C. Hu (eds.). Fruit crops: diagnosis and management of nutrient constraints. Elsevier, Amsterdam. Doi: https://doi.org/10.1016/B978-0-12-818732-6.00005-8

Rizzardi, M.A., N.G. Fleck, R.A.Vidal, A. Merotto Jr., and D. Agostinetto. 2001. Competição por recursos do solo entre ervas daninhas e culturas. Cienc. Rural 31(4), 707-714. Doi: https://doi.org/10.1590/S0103-84782001000400026

Rodríguez-García, D. and J. Vargas-Rojas. 2022. Efecto de la inoculación con *Trichoderma* sobre el crecimiento vegetativo del tomate (*Solanum lycopersicum*). Agron. Costarr. 46(2), 47-60. Doi: https://doi.org/10.15517/rac.v46i2.52045

Stasińska-Jakubas, M. and B. Hawrylak-Nowak. 2022. Protective, biostimulating, and eliciting effects of chitosan and its derivatives on crop plants. Molecules 27(9), 2801. Doi: https://doi.org/10.3390/molecules27092801

UPRA, Unidad de Planificación Rural Agropecuaria Colombia. 2023. Resultados preliminares evaluaciones agropecuarias, diciembre de 2023. Evaluaciones agropecuarias municipales. Bogota.

Ugarte-Barco, F.A., I.A. Zhiñin-Huachun, and R. Hernández-Pérez. 2022. Influencia de bioestimulantes sobre caracteres morfológicos y agroquímicos del banano (*Musa* AAA cv. Williams). Terra Latinoam. 40, e1456. Doi: https://doi.org/10.28940/terra.v40i0.1456

Vasconcelos, A.C.F. and L.H.G. Chaves. 2020. Biostimulants and their role in improving plant growth under abiotic stresses. In: Mirmajlessi, S.M. and R. Radhakrishnan (eds.). Biostimulants in plant science. IntechOpen. Doi: https://doi.org/10.5772/intechopen.88829

Wagner-Medina, E.V., J.A. Valencia, Á. Caicedo, and J.F. Hernández. 2023. Manual técnico para producir semilla asexual de calidad de plátano cv. Dominico Hartón por macropropagación. Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Mosquera, Colombia. Doi: https://doi.org/10.21930/agrosavia.manual.7406139

Yasmeen, R. and Z.S. Siddiqui. 2017. Physiological responses of crop plants against *Trichoderma harzianum* in saline environment. Acta Bot. Croat. 76(2), 154-162.