# Physiological response induced by biostimulants on plantain plants (*Musa* AAB) under *Ralstonia solanacearum* race 2 stress[1]

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## ABSTRACT

*Ralstonia solanacearum* race 2 (Rs) is the causal agent of Moko, one of the most limiting diseases of plantain. This study aimed to determine if the preventive application of salicylic acid (SA), silicon dioxide (Si), *Bacillus amyloliquefaciens* (Ba), and *Bacillus subtilis* (Bs) reduce the disease development and mitigate the plant physiological damage caused by Rs. For this, plantain plants cv. Hartón (*Musa* AAB) at nursery stage were established in a split-plot design and treated with biostimulants before the inoculation with Rs. Disease development and plant physiological variables were evaluated post-inoculation. Application of SA and Si on inoculated plants improved the relative water content, quantic yield of the photosystem II, electrons transport rate, gas exchange, and promoted dry biomass partition to the roots and a higher roots elongation. Plants treated with these biostimulants showed the lowest disease degree in comparison to inoculated control plants. Moreover, non-inoculated plants treated with Si and Bs significantly improved their photosynthetic capacity, biomass accumulation, and root elongation compared to non-inoculated control plants. Results suggest that preventive application of SA and Si reduces the Moko disease development whereas Si and Bs improve the physiological features of the plants.


**Keywords:** plant physiology; resistance inductors; gas exchange; roots; moko.

## INTRODUCTION

*Ralstonia solanacearum* is the causal agent of vascular wilt in multiple hosts including economically important species (Genin, 2010). It has been considered one of the most destructive pathogens difficult management (Aloyce *et al.,* 2017). This soil borne bacteria habitually infects the plant, colonizes the root surface, and invades it (Xue *et al.,* 2020). Once the vascular system is colonized, it blocks the water transport both due to a high bacterial charge and exopolysaccharides secretion, causing progressive wilt in the plant and death (Zheng *et al.,* 2017). The bacteria multiply massively in the xylem vessels, occludes them, and induce a progressive reduction of the stomatal conductance and photosynthetic rate (Fan *et al.,* 2018), which may generate an energy excess and affect the photosystem II (PSII) integrity irreversibly, like severe water stress condition (Dalal & Tripathy, 2018). Besides, Rs infection modifies the host roots morphology, inhibiting primary roots growth, and promoting lateral roots and root hair growth (Zhao *et al.,* 2019). Different strategies to manage this disease have been evaluated.

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These include the use of resistant varieties, chemical and biological control and, more recently, the use of biostimulants (Vargas *et al.*, 2023; Wang *et al.*, 2023).

Biostimulants are products that promote metabolic processes with the aim of improving plant growth, development, and yield, helping them to overcome biotic and abiotic factors by inducing multiple responses (Parađiković *et al.,* 2019). These products with biostimulant activity have been reported as well as defense elicitors that allow plants to endure the attack of biotic agents (Du Jardin, 2015; Parađiković *et al.*, 2019). Biostimulants are available in a variety of formulations and ingredients. Accordingly, they can be grouped into the following categories: humic and fulvic acids, algae and microalgae extracts, animal and plant protein hydrolysates, arbuscular mycorrhizal fungi, N-fixing bacteria, plant oils, inorganic compounds, hormones, chitosan and growth-promoting rhizobacteria (PGPR) (Rouphael & Colla, 2020; Du Jardin, 2015, Calvo *et al.*, 2014). Biostimulants has shown to activate different defense mechanisms against *R. solanacearum* in tomato, pepper, and tobacco (Narasimhamurthy *et al.,* 2019; D'Addazio *et al.,* 2020), reducing the disease degree, bacterial growth and consequently, promoting a better water status, photosynthetic activity and therefore, reduce the infection impact on the host (Fan *et al.,* 2018; Sayago *et al.,* 2020). Nevertheless, there are no reports about the effect of these products on disease development and Musaceae physiology infected by *R. solanacearum*.

In Musaceae, the disease caused by *R. solanacearum* race 2 (Rs), is known as Moko and it is widely distributed in plantain and banana producer countries (CABI, 2020). It is considered one of the most limiting diseases in these productive systems (with losses reported from 70 to 100% of the established area), not only for its high severity but also for the quarantine status that holds in some countries (Ramírez *et al.,* 2020). Once the symptomatic plants are detected in Colombia, the focus must be eradicated, including symptomatic and asymptomatic plants located in a radio of 5 m (Álvarez *et al.,* 2010). Recommendations for its management are limited (Ceballos *et al.,* 2014; Ramírez *et al.,* 2020). Studies with biostimulants used for its control have focused on quantifying the reduction of the disease, but not on researching their effects on plant physiology (Ramírez *et al.,* 2020). The objective of this work was to assess the physiological response of plantain plants cv. Hartón treated with biostimulants to reduce Rs infection, and to evaluate the potential of these products to delay the disease symptoms during the nursery stage.

## MATERIALS AND METHODS

### *Plant material and Experimental site*

Two-and-a-half-month-old plantain plants cv. Hartón (*Musa* AAB) of *in vitro* origin were transplanted into capacity 10 kg plastic bags, containing a mixture of soil and rice husk (3:1 v/v). They were kept in a greenhouse under semi-controlled conditions, in the Research Center La Libertad (Corporación Colombiana de Investigación Agropecuaria, Agrosavia; Villavicencio, Colombia). The average daily temperature was 30 ± 4 °C and the relative humidity of 75 ± 5%. Plants were manually irrigated to maintain the soil at field capacity (which was determined by gravimetry), and the soil moisture content was daily monitored with a Moisture Meter Device (type HH2, Delta-T devices Ltd.).

### *Inoculum production of Ralstonia solanacearum*

A high virulent strain of Rs race 2 (GRsDFII) previously isolated from symptomatic plantain plants and preserved in the microorganism collection of Agricultural Microbiology Laboratory at Agrosavia (Mosquera, Cundinamarca), was used to infect the plants. Bacterial inoculum was increased in nutrient broth from cultures of 48 hours grown on plates with Kelman's TZC agar. It was incubated at ± 28 °C at 150 rpm for 36 hours. Cell growth was measured by spectrophotometry at 600 nm and adjusted to an optical density of 0.3 equivalent to 1 x $10^8$ colony forming unit (CFU) $mL^{-1}$ concentration (He *et al.,* 1983).

### *Experimental design*

Commercial biostimulant products were applied in two-and-a-half-month-old plants. The products were a solution of Salicylic Acid 15% (SA), *Bacillus amyloliquefaciens* (Ba, 1x$10^8$ CFU $mL^{-1}$, Baliente), *Bacillus subtilis* (Bs, 5x$10^9$ CFU $mL^{-1}$ Bactox), and silicon dioxide (Si, Tierra de diatomeas), to 1.5 mL $L^{-1}$, 2.5 mL $L^{-1}$, 1.25 mL $L^{-1}$, and 8 g $L^{-1}$ respectively according to commercial recommendation. SA was applied on leaves, Ba and Bs by pouring over the substrate, and Si on the substrate and leaves at 100 mL $plant^{-1}$ 15, 8, and 1 day before the inoculation, and four days post inoculation (dpi). Plants were infected by direct injection into the corm of the sucker with 3 mL of bacterial suspension at 1x$10^8$ CFU $mL^{-1}$.

The experimental design was split-plot with main plots arranged as a randomized complete block and repeated measurements during the time. The main plot was the inoculation factor (inoculated: I, and non-inoculated: NI), and subplots were the biostimulants (four products and the control, non-inoculated control, NIC, and inoculated control, IC). Each treatment had four repetitions, each one consisting of nine plants. Four repeated measurements were done for the evaluated variables: 1 dpi, 6 dpi (symptoms start, when more than 50% of the inoculated control plants showed symptoms), 8 dpi, and 10 dpi, (when more than 50% of the IC plants showed the maximum disease grade), except for dry biomass and root length variables, which were evaluated one time (10 dpi).

### *Disease and physiological data*

The disease was evaluated using the modified severity scale purposed by Ceballos *et al.,* (2014) (Table 1). The severity measurements over time were used to calculate the area under the disease progress stair, AUDPS (Simko & Piepho, 2012).

*Gas exchange and chlorophyll fluorescence*: theses variables were evaluated with a portable photosynthesis system Li-6400XT (Licor Inc., Lincoln, NE, USA). The registered parameters were photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs). Readings were taken between 9:00 and 11:30 am; the flow density of photosynthetic photons was adjusted to 1200 μmol $m^{-2}$ $s^{-1}$ according to the results of light saturation curve, and $CO_2$ concentration at 400 μmol $mol^{-1}$. In the second case, the measurements were registered at night in the same plants with a camera Li-6400-40. Electrons transport rate (ETR) and quantic yield of the PSII (ΦPSII) were registered. Measurements were taken in the third-youngest leaf completely expanded.

*Chlorophyll relative content*: To determine this parameter a portable chlorophyll meter SPAD-502 (Konica-Minolta) was used. The chlorophyll index (SPAD units) registered by plant corresponds to the average of three readings taken in the same leaf in the middle third.

*Relative water content:* it was determined according to Barrs & Weatherley (1962), using the following formula: $RWC = [(FW - DW) / (TW - DW)] \, x \, 100$, where: FW: fresh weight; TW: turgid weight and DW: dry weight. Four slides obtained from the third-youngest leaf per treatment were taken in each sampling.

*Dry biomass accumulation:* Fresh samples of each plant organ (leaves, pseudo-stem, corm, and root) of 10 dpi, were weighted in the balance and dried in a stove at 65 ºC for 72 hours. Finally, the total dry weight per plant was measured.

*Root length:* Complete roots of four plants per treatment were separated from the corm at 10 dpi. They were carefully washed and preserved in FAA solution (10% formaldehyde, 5% glacial acetic acid, 55% ethyl alcohol, and 30% water). The length was measured for two root types classified according their diameter: < 5.00 and > 5.00 mm corresponding to lateral and adventitious roots respectively (Draye *et al.,* 2005). The image analysis program WinRhizo (Régent Instr. Inc., Quebec, Canada) was used along with computer equipment connected to a root scanner Epson Perfection V800 Photo.

### *Statistical Analysis*

Mixed model analysis was used to determine the significance of the factors on the evaluated variables. The normality and homoscedasticity assumptions were proved using variance functions to model the variance structure inside the groups when they were found heterogeneous. The factors considered in the model were: inoculation, treatments, time (as fixed effects), and blocks (as randomized effect). The final model was selected considering the *top-down* and *backward* methodology (Zuur *et al.,* 2009), and Akaike and Bayesian information criterion. Afterward, according to the results of multiple comparisons, differences were considered significant when $p < 0.05$ according to DGC (Di Rienzo, Guzmán and Casanoves) test, always considering the obtained order major interaction. The variables that did not contemplate repeated measurements in the time (dry biomass and root length) were analyzed

**Table 1:** Moko disease severity scale modified from (Ceballos et al., 2014) in plantain

| Grade | Symptoms |
|---|---|
| 0 | No visible symptom |
| 1 | Loss of turgor and plant flabby appearance. |
| 2 | Slight wilting and leaves with loss of dark green color. |
| 3 | Advanced wilt and initial yellowing of leaves. |
| 4 | Whole plant wilting and yellowing leaves with small necrotic lesions. |
| 5 | Whole plant wilting twisted and necrotic leaves. |

with a two-way ANOVA; differences among treatments were determined through a Tukey test ($p < 0.05$) with Bonferroni adjustment. For the analysis, the programs R (v. 3.6.0) and InfoStat v. 2017 (Universidad Nacional de Córdoba, Córdoba, Argentina) were used.

## RESULTS

### *Effect of biostimulants on the disease development*

The severity evaluation of Moko disease in inoculated plantain plants showed significant differences in the Time-Biostimulants interaction ($p < 0.05$). The highest reductions in severity were reached with the SA and Si treatments at 22.2% and 19% respectively, while the highest disease degree at the end of the trial was obtained in plants inoculated control (Figure 1). The first symptoms were registered at 6 dpi when more than 50% of the plants showed symptoms, and they reached the maximum severity degree at 10 dpi. Plants treated with Bs, and Ba developed symptoms at an intermediate level, and no symptoms of the disease were observed in non-inoculated control plants.

### *Effect of the Rs infection on gas exchange and RWC parameters*

The results of the statistical analysis showed a significant effect ($p < 0.05$) in the interaction of Inoculation-Biostimulants-Time factors on the gas exchange (Figure 2A-C) and RWC variables (Figure 2D). This interaction was observed from 1 dpi, although, it was more evident from 6 dpi when the first infection symptoms were shown (Figure 2). From this point, values of the gas exchange and RWC variables in non-inoculated plants were stable or improved depending on the biostimulant applied, compared with inoculated plants in which their values were reduced (Figure 2). Non-inoculated plants treated with Si and Bs increased 84% their photosynthetic activity at 6 dpi while NIC plants and those treated with SA, and Ba increased by 41%. Inoculated plants treated with Ba and Bs reduced it by 38% at 6 dpi, although inoculated plants treated with Si and SA increased it by 7%.

The progression of the disease gradually reduced the gas exchange capacity, mainly in IC plants in which Pn values of -0.33 µmol $CO_2$ $m^{-2}s^{-1}$ were registered at 10 dpi, when the average disease degree was 4.6, while treated plants with SA and Si maintained the photosynthetic rate at 3.1 and 2.4 µmol $CO_2$ $m^{-2}s^{-1}$ respectively. Contrarily, non-inoculated plants, stimulated with Si and Bs reached the best photosynthetic activity along the time reaching an increase of 62% and 51% respectively. On the other hand, NIC, SANI, and BaNI kept their constant activity (average value of 8.43 µmol $CO_2$ $m^{-2}$ $s^{-1}$) (Figure 2). The trend was similar in the exchange variables, Gs and Tr for all the treatments (Figure 2B and 2C). In plants without pathogen, Bs and Si treatments stimulated Pn, Gs, and Tr, which was evident by the major slope inclination.

Significant variations in the RWC of non-inoculated plants were not observed. However, in inoculated plants with Rs, the RWC was significantly reduced in all treat-

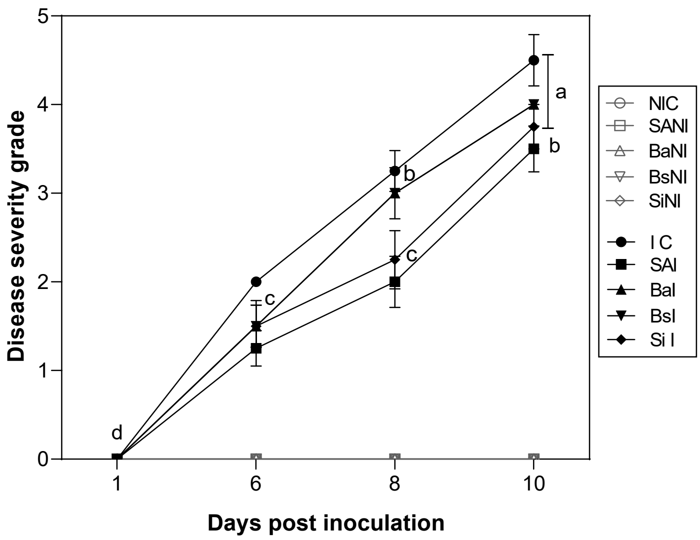


**Figure 1**: Disease severity grade of plantain cv. Hartón treated with four biostimulant products and infected with *R. solanacearum*. NI: non-inoculated; I: inoculated; C: control; SA: Salicylic acid; Ba: *B. amyloliquefaciens*; Bs: *B. subtilis*; Si: silicon dioxide (n = 4). Interaction between inoculation, treatment and time was significative ($p < 0.05$).

ments. NIC at 1 dpi, without apparent Moko symptoms, showed an average reduction of 4% compared with other treatments, pointing out an early effect of the Rs colonization on the water state of the plant. The RWC was reduced to critical values of 51% when the disease progressed. Si followed by SA preserved a better RWC in inoculated plants with average values of 73.6% and 71.4% respectively at 10 dpi. Plants treated with Ba and Bs had similar behavior to IC until 8 dpi since in the last RWC evaluation the IC dropped differential (until 51%).

### *Effects of Rs infection on the chlorophyll fluorescence*

The interaction of inoculation and treatments during the time was significant on ΦPSII and ETR ($p < 0.05$). Non-inoculated plants kept stable values in the three variables during the experiment, although ETR increased (13.2%) from the first to the sixth dpi, and then it was stabilized. On the other hand, inoculated plants showed significant reductions in the integrity of the Photosystem II (PSII) during the time (Figure 3A-3B). SA and Si biostimulants were able to significantly reduce the effect caused by Rs on the PSII compared to Ba and Bs, while IC showed the lowest values. Reduction between the first and the last evaluation in IC plants was 37.7% and 50.1% for ΦPSII and ETR respectively, being ETR the more sensitive variable to the effect caused by Rs since its drop was observed from 6 dpi.

### *Effect of biostimulants on the dry matter accumulation and root length in inoculated and non-inoculated plants with Rs*

Differences in the inoculation-treatment interaction were found in the dry biomass (DM) accumulation at the end of the experiment. Infection with Rs significantly reduced DM, while non-inoculated plants kept or increased it (depending on the biostimulant used) compared to NIC (Figure 4A). The highest DM accumulation in inoculated plants was achieved with the use of SA, 35.4 g, 47% more than IC, followed by Si and Bs, which increased it by 32%. The lowest values were obtained by IC plants that reduced

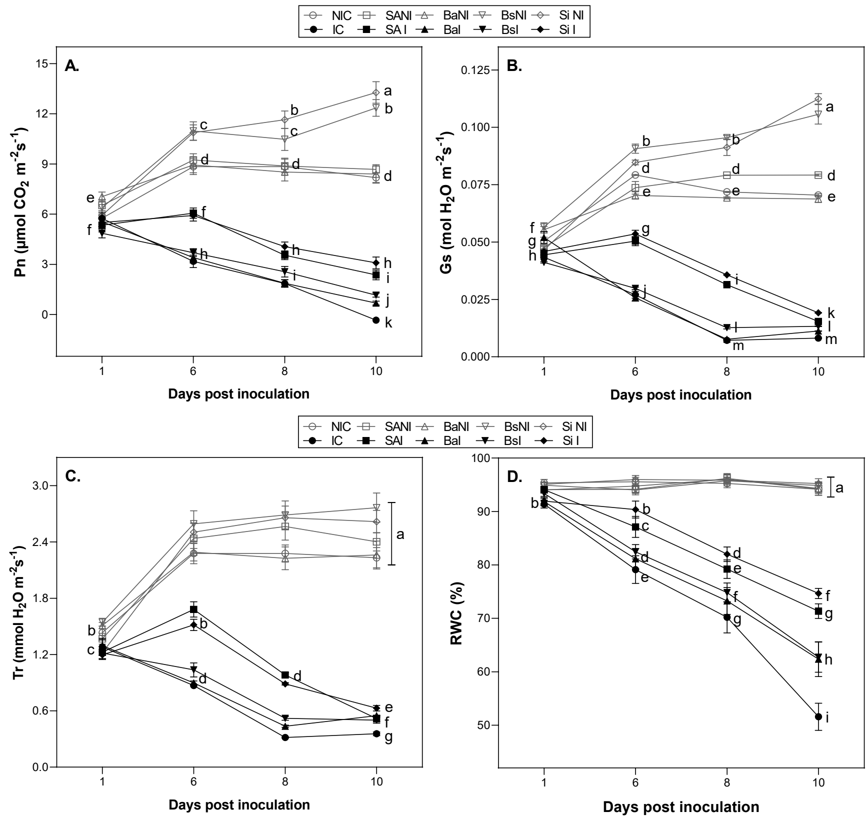


**Figure 2**: Changes in photosynthesis rate (Pn, A), stomatal conductance (Gs, B), transpiration rate (Tr, C) and relative water content (RWC, D) of plantain cv. Hartón treated with four biostimulant products, inoculated and non-inoculated with *R. solanacearum*. Grey lines: non-inoculated (NI); black lines: inoculated plants (I). Legend: C: control; SA: Salicylic acid; Ba: *B. amyloliquefaciens*; Bs: *B. subtilis*; Si: silicon dioxide (n = 4). Interaction between inoculation, treatment and time was significative ($p < 0.05$) according to DGC's test.

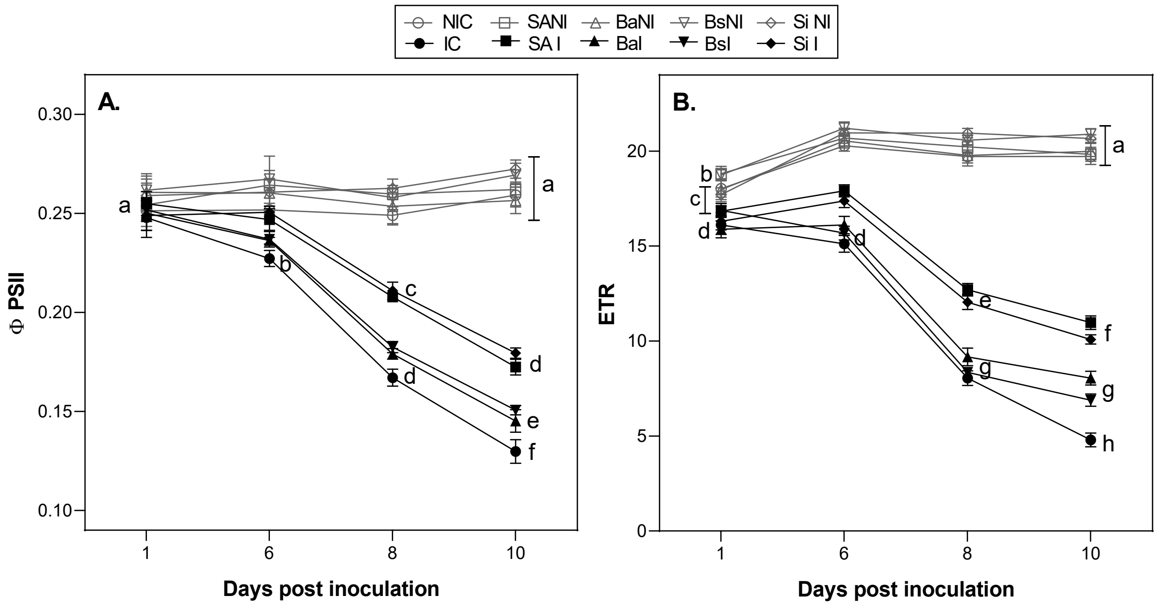


**Figure 3**: Changes in PSII actual photochemical quantum yield (ΦPSII, A) and electron transport rate (ETR, B) of plantain plants cv. Hartón treated with four biostimulant products, inoculated and non-inoculated with *R. solanacearum*. Grey lines: non-inoculated (NI); black lines: inoculated plants (I). Legend: C: control; SA: Salicylic acid; Ba: *B. amyloliquefaciens*; Bs: *B. subtilis*; Si: silicon dioxide (n = 4). Interaction between inoculation, treatment and time was significative ($p < 0.05$) according to DGC's test.

their DM by 33% compared to NIC. Bs and Si treatments in non-infected plants improved the dry matter accumulation (18% and 16% more than the control), while NIC, SA, and Ba presented no differences.

Dry matter distribution among organs (Figure 4A) varied with the interaction of biostimulant-inoculation 10 dpi. NIC plants and those treated with SA had a higher DM accumulation in leaves, while the ones treated with Ba, Bs, and Si had it in the pseudostem. In inoculated plants, Rs modified the total accumulated DM and its distribution. The partition of assimilate to the root increased in all treatments, with a higher percentage for SA (2.9%). This treatment achieved the conservation of the higher amount of DM in plants and increased the partition to the pseudostem (5.3%) and reduced it to the leaves (10.5%). While in plants treated with Si, the distribution to the leaves increased and reduced the distribution to the pseudostem.

Dry biomass accumulation in roots and root length (RL) were affected by inoculation and biostimulants (Figure 4B, 4C). Inoculated plants treated with SA accumulated DM in roots with similar values to the best treatment in healthy plants (BsNI). Moreover, SA induced the highest total RL (4667.6 cm) at 10 dpi, and higher growth in lateral roots as a response to the stress by Rs (39% compared to IC), followed by Si treatment (Figure 4D). Inoculated plants showed higher lateral RL than non-inoculated plants. However, among the non-inoculated plants, Bs and Si induced the highest root length of 28% and 18% compared to the control plants with significant differences. Rs maintained the adventitious root length in the lowest value in IC plants, followed by treated plants with biostimulants, which showed no significant differences among them.

## DISCUSSION

Biostimulants are products that are applied to the plants with the purpose of improving its yield, quality, and productive efficiency. Although, they also promote the biotic and abiotic stress tolerance through complex changes regulated by physiological, biochemical, and molecular processes (Du Jardin 2015; Parađiković *et al.,* 2019). In this work, it was found that the preventive application of SA and Si biostimulants on Hartón plantain plants infected with Rs delayed the disease development and mitigated its effect improving the physiological response in plants, likely through defense response induction. Thus, the hydric status, gas exchange, chlorophyll fluorescence, total biomass accumulation, and roots growth were superior compared to IC plants. In non-inoculated plants, biostimulants of biological origin (Bs) and inorganic origin (Si) significantly increased the photosynthetic activity, total and roots dry biomass accumulation.

According to Choi *et al.* (2013), the occlusion caused by vascular pathogens induces a hydric stress condition in the plant due to imposed limitations in the water transport

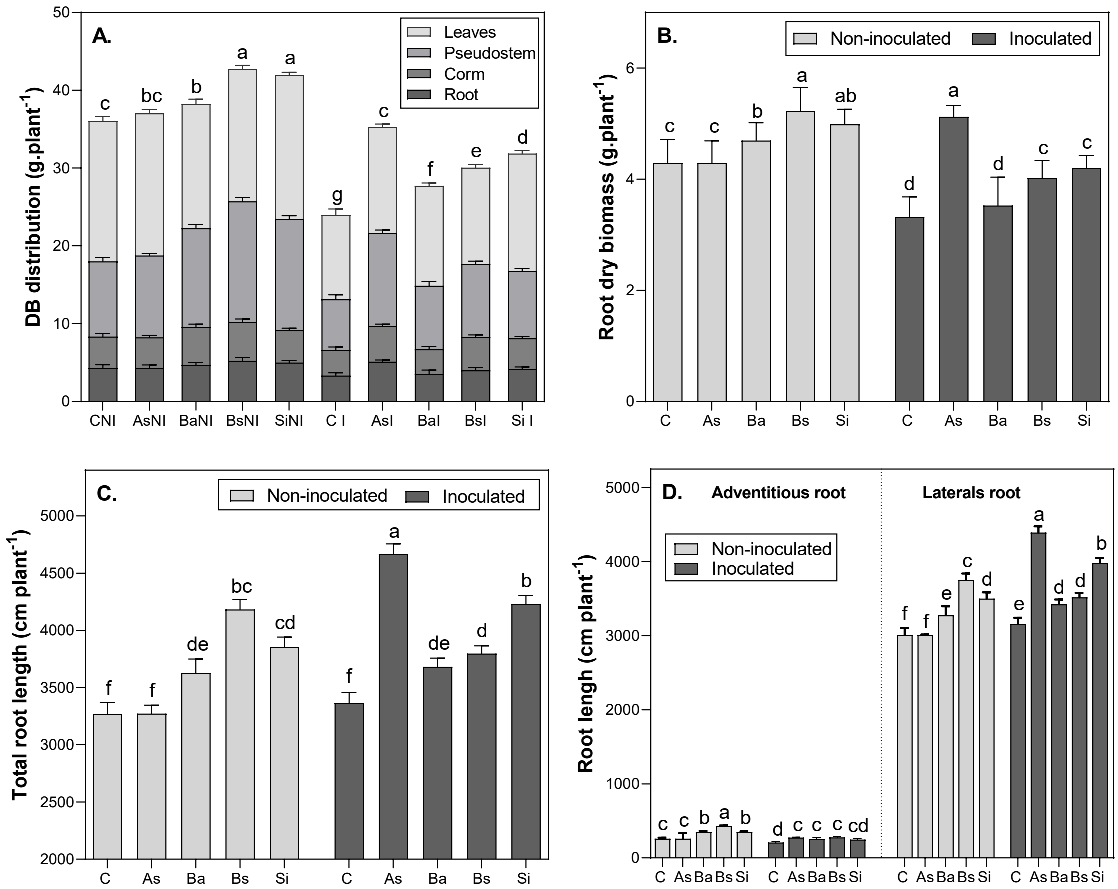


**Figure 4**: Distribution of dry biomass, DB, among organs (different letters indicate significant difference in total biomass accumulation between treatments according with Tukey test ($p < 0.05$) (A)), root dry biomass (B), total root length (C), laterals and adventitious root length (D) of plantain plants cv. Hartón treated with four biostimulant products, inoculated and non-inoculated with *R. solanacearum*. C: control; SA: Salicylic acid; Ba: *B. amyloliquefaciens*; Bs: *B. subtilis*; Si: silicon dioxide (n = 4). Interaction between inoculation, treatment and time was significative ($p < 0.05$) according to DGC's test.

from the roots to the leaves. Water restriction reduces the stomatal conductance (Gs) and, consequently, the $CO_2$ assimilation rate is affected (Wang *et al.,* 2018). Even though there are no reports of Musaceae species treated with biostimulants and inoculated with Rs. In this work it was found that the impact caused by the pathogen on the plant physiology was associated to a hydric restriction condition, as it is suggested by the evaluated variables, however, this impact was mitigated by the Si and SA spraying.

According to results, SA reduced the negative effects caused by Rs, it favors physiological responses like the photosynthetic activity, RWC, dry biomass accumulation, root length, and contributes to delaying the disease progress. Several authors have pointed that the SA application can trigger systemic disease resistance that induce reinforced cell walls, oxidative burst and gene expression regulation in different plant species interacting with diverse pathogens, in addition, SA inhibits Rs growth and represses several virulence factors (Vailleau & Genin, 2023; Chávez-Arias *et al.,* 2020). The RWC was best preserved in SA treated plants, likely by the promotion of the osmotic adjustment that allowed the plant to keep a better turgor (Mimouni *et al.,* 2016). Therefore, the application of this treatment allowed the reduction of the pathogen impact on the gas exchange. This is the first report of the physiological behavior of plantain plants treated with biostimulants and infected with Rs. Similar results in golden berry inoculated with *Fusarium oxysporum* f. sp. *physali* (vascular pathogen) treated with SA showed improved Gs, chlorophyll fluorescence and growth (Chávez-Arias *et al.,* 2020).

Likewise, SA improved the total DM accumulation and promoted lateral roots length compared to IC plants and disrupted the assimilates distribution in a higher percentage to the root and pseudostem. In addition, SA regulates plant growth through mediation of cell division and expansion (Li *et al.,* 2022). Studies in *Arabidopsis thaliana* showed changes in the root architecture (lateral roots formation and root growth inhibition) after Rs inoculation as a strategy to improve its colonization (Lu *et al.,* 2018; Zhao *et al.,* 2019). This change in the architecture may also be explained as a secondary effect in the plant caused by the increased levels of auxin promoted by Rs and not by the colonization effect. Caldwell (2016) reported that these modifications in the tomato rooting system may be associated with plant defense mechanisms as a strategy to improve their access to water and nutrients, instead of colonization strategies by Rs. Features related to the root morphology have been associated with Rs tolerance in some hosts (Lu *et al.,* 2018; Nansamba *et al.,* 2020). In this work the best lateral root development was related to the mitigation of disease symptoms induced by SA and Si treatments. However, there are no additional reports in Musaceae, so the research in this topic should be continued.

Silicon dioxide also mitigated the effect of Rs on physiological parameters of the plant. Silicon has been recognized as reducing damage in plants under biotic and abiotic stresses (Zhang *et al.,* 2018). In this work, a delay of wilt symptoms was found in plants treated with Si, which showed the best levels of RWC. Preservation of the water status may be related to the basal defense response at the cell wall level that allows water loss reduction, accompanied by a multiple hormone response (that involves SA and jasmonic acid/ethylene) (Song *et al.,* 2014; Jiang *et al.,* 2019).

Si application partially regulates the expression of some genes related to the photosynthesis in water deficit conditions (Zhang *et al.,* 2018), increases transcription of genes involved in the PSII efficiency and the ETR (Song *et al.,* 2014). These effects were found in this research where Si improved (along with SA) the gas exchange measured as Pn, Gs and Tr in comparison with Ba and Bs treatments. In addition, Si kept the levels of ETR until advanced stages of the disease (11.5 to 10 dpi), showing its positive effect on the plant physiological parameters. Similar trends were found in pepper plants treated with Si and infected with *Fusarium solani* f. sp. Piperis (D'Addazio *et al.,* 2020).

Ba and Bs treatments did not have a significative effect on the symptoms development compared to IC at the end of the experiment. A similar behavior was obtained in gas exchange analysis. Variable results have been reported with the use of biostimulants with bio-controlling capacity. It is the case of *B. amyloliquefaciens* strains evaluated to control Rs in tomato, which had the best bio-control efficiency compared with another microorganism (Singh *et al.,* 2016). The effects of this PGPR in the vascular wilt control were associated with its antibacterial activity in the soil rhizosphere and its high capacity of competitive exclusion through colonization and formation of biofilms in the root surface (Tan *et al.,* 2016; Elsayed *et al.,* 2020). The lack of biocontrol with Ba and Bs in our results may be attributed to the inoculation method of Rs, which prevented that Ba and Bs compete with Rs to colonize the rhizosphere (Elsayed *et al.,* 2020).

Biostimulants applied to non-inoculated plants, particularly Si and Bs, induced the best response in gas exchange (Pn, Gs and Tr) and dry matter compared to control plants. Similar increase was reported in treated rice plants versus no treated plants (Song *et al.,* 2014), and in plantain plants using these compounds (Mateus-Cagua & Rodríguez-Yzquierdo, 2019). Other studies reported an increase in the biomass accumulation, length and volume root induced by the treatment in non-stressed plants (Zhang *et al.,* 2019). Bs promoted a better biomass accumulation and root development (higher adventitious and lateral roots length among the evaluated treatments), which could be related to the synthesize and release of auxin, a hormone involved in cell division and tissue differentiation (Sayago *et al.,* 2020). Therefore, Bs application can stimulate the root growth for a better exploration of water and nutrients and prepare it for a stressing event (Sayago *et al.,* 2020). During the evaluation period, the water status measured through the RWC was stable as well as the chlorophyll fluorescence.

## CONCLUSION

Bs and Si biostimulants favor the physiological processes in non-inoculated plantain plants in the nursery stage.

Both Si and SA delay appearance disease symptoms and maintain a better water status in the plant during the vascular infection caused by Rs when they are applied preventively. So that these treatments may be used at nursery plants production as growth promoters and resistance inductors.

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Authors declare there is no conflict of interests in carrying out the research and publishing this manuscript.

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