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Growth Promoting Rhizobacterium Effects on *Coffea arabiga* Scion onto Robusta Rootstock

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Abstract

Context: No reports about the response of the main *Coffea arabica* L. genotypes grafted onto *Coffea canephora* in the presence of plant growth promoting rhizobium have been found yet.
Aim: To evaluate the effect of *Rhizobium alamii* Rpr2 on *C. arabica* L. development while grafted onto *C. canephora* (Robusta).
Methods: The trial was conducted under a randomized block design, with a 4x2 factorial arrangement, in four replicas. The factors were four *C. arabica* L. genotypes. (Isla 6-14, Isla 5-15, Isla 6-11, and San Ramón), and two rhizobium application levels (with rhizobium or without it). The days to inosculation were evaluated, along with the percentage of graft inosculation, graft height, graft stem height, number of graft leaf pairs, foliar area, dry leaf mass, root growth, root volume, and root dry mass.
Results: The results showed the interaction between the grafted *C. arabica* species, and strain *R. alamii* Rpr2. The inoculated scion Isla 6-14/Robusta showed a better response than the other treatments and the control, which makes it a suitable variant for coffee growing.
Conclusions: The utilization of plant-growth promoting bacteria is an effective alternative for grafting stimulation and development, which might lead to greater availability of plantlets ready for large-scale planting in the field.

Keywords: *genotypes, rhizobium, plant nursery.*

Introduction

Proper coffee tree handling must rely on nursery management, especially if vigorous and healthy plants must be used for the definitive plantation (Valarezo et al., 2021). Tolerant genotypes or high-value variety grafting onto resistant rootstocks are grown to prevent common coffee diseases (Santos et al., 2017).

The hypocotyl grafting consists of using a stem (rootstock) of *Coffea canephora* P. (nematode tolerant), and a *Coffea arábigo* L. bud (which offers higher physical and organoleptic quality). It is a low-cost and relevant solution that has been previously tested to control pests and diseases affecting plant roots, to which the rootstock is resistant (Reyes et al., 2016; Cantos et al., 2018).

Grafting onto the Robusta pattern, using Arabico scions produces nematode and drought-resistant plants (Tigua, 2019). This grafting technique includes different factors that mediate the behavior of the grafting stock and graft growth (Borjas-Ventuea et al., 2018).

This practice, particularly onto a vigorous *C. canephora*pattern, with the suitable variety, may also be an alternative to improve coffee productivity and cost-efficiency (Julca et al., 2018). Then, a broad range of variety combinations of rootstocks/scions or buds must be selected to produce a reliable and compatible graft.

The scion/stock interaction depends on several different factors, such as the plant material used for grafting (Barbosa et al., 2014). Because genetic factors are involved, the plant response is varied and unique, depending on the local characteristics (interaction with the environment), in addition to the fact that plant growth and development are regulated by phytohormones (Cantos et al., 2018).

Some rhizobacteria have the capacity to produce phytohormones, one of the most widely studied mechanisms associated with plant growth promotion. In that sense, Parray et al. (2016) referred to the usefulness of *Rhizobium* spp. for phenological development and the productivity of different crops due to their potential emission of plant-growth-promoting substances.

Coffee cultivation in Cuba rests on the generation of new genetic material, and on the implementation of new and efficient propagation methods. Despite this fact, the new coffee genetic material generated in Cuba has not been evaluated in terms of their behavior in a grafting-based propagation system, and against the presence of *Rhizobium* bacteria. Hence, there is no basic information to improve management. Accordingly, the purpose of this paper was to evaluate the response of new coffee cultivars by grafting scions onto *C. canephora*, exposed to the promising rhizobium in the nurseries.

Materials and methods

The experimental work was done in the 2020-2021 period, at the technical nursery of La Caoba Basic Production Cooperative (UBPC), which belongs to the San Luis Agroforestry Company, in Cuba. The *C. canephora* (Robusta) seeds for the grafting stock and the *C. arabica* seeds for grafting, were provided by the UBPC's germplasm bank. Isla 6-14, Isla 6-11, Isla 5-15, and San Ramón.

A *Rhizobium* sp (Rpr2) isolate was used. It was isolated from the rhizosphere of rice plants (*Oryza sativa* L.) cultivar INCA LP-5, in Cuba (Hernández & Nápoles, 2017). Erlenmeyer flasks (250 mL), containing 50 mL liquid medium of mannitol yeast (MY) were used to obtain the pre-inoculum. The *Rhizobium* sp (Rpr2) isolate was kept at 4 ºC in test tubes containing mannitol yeast agar medium (MY) (Vincent, 1970). The cell concentration was adjusted to $1x10^{10}$ cells $mL^{-1.}$

The study used a completely randomized block design (CRBD) in a common 4x2 factorial array, and 4 replicas, totaling 32 experimental units. The factor I: Grafts (Isla 6-14/Robusta, Isla 6-11/Robusta, Isla 5-15/Robusta, and San Ramón/Robusta), and Factor II: Inoculation of the rhizobium strain (presence and absence).

Following the split method recommended for this crop (Villain, 1994) the root system of the grafts selected was treated with or without the inoculum. The grafts were dipped in a glass containing 1mL of the medium diluted in 9 mL of water, for one hour. The final volume was 10 mL, at room temperature in the shade, or in the same volume of water to perform the control treatment (Cisneros et al., 2017). It was plated on poly-foam trays containing sterile wet river sand. The trays with the grafts were placed in the adjustment chamber at ±27 ºC, and 75/85% relative humidity, for 30 days. Moisture was kept using frequent irrigation, to maintain the field capacity.

The evaluations performed for the next 30 days following the application of the treatment consisted of: Days to inosculation (DI, days), observed in days lapsed from grafting, and until over 50% of the plantlets showed some inosculation, and the inosculation percentage (IP, %) was obtained by quantifying (inosculated scions / No. of total grafts) x 100 (Cantos et al., 2018).

Before transplanting to the propagator, 1 kg black polyethylene bags were filled with the substrate made of a mix containing lixiviated red ferralitic soil (Hernández-Jiménez et al., 2015), and sugarcane residues, as organic matter, using a 2:1 (v:v) proportion. The substrate was completed with phosphoric fertilization (triple superphosphate) $[Ca(H_2PO_4)^2*H_2O]$, at a rate of 10.9 kg m^3 of the mix. Before planting, the soil was slightly irrigated. The weeds and obstacles in the plantation area were cleaned manually to enable aeration inside the bags.

The evaluations were made 210 days after the treatment (DAT), and consisted of analyzing 20 plants from every treatment, totaling 160 plants. The grafting height (GH, cm), grafting diameter (GD, mm), number of leaf pairs in the grafts (LP, pair), root length (RL, cm), foliar area (FA, cm^2) were evaluated as well, using the following formula: FA= [(L x W) x 0.64], where L is leaf length and W is the leaf width. The dry biomass was collected by weighing the foliar part (DMF, g), and the root part (DMR, g) using an analytical balance with previous drying in a Mermet forced air stove, at 75 - 80 °C, to achieve constant weight (Roberts et al., 1988). The root volume (RV) was calculated based on the Archimedes principle, using a 500 ml tube with 200 mL distilled water. The roots were introduced to estimate their total volume per plantlet through water displacement (Córdoba-Rodríguez et al., 2011).

$VR = (. _2 +) -. _2$

Data normality was corroborated through the Kolmogorov-Smirnov test, while the variance homogeneity was measured by the Levene test. Then, the analyses of variance were performed, and the

different means were compared through the Tukey's test (95%). The variable correlations were determined, and a network of correlations was constructed using the Rbio program (Bhering, 2017). The correlations above 0.65 appeared on the network, in green (positive) and red lines (negative).

Results and Discussion

The analysis of the assay data permitted the detection of a significant interaction between the two factors (grafting x inoculation) for all the variables evaluated (Table 1). Likewise, the efficiency of the rhizobium strain inoculated was dependent on the Arabic cultivar type grafted onto the Robusta pattern.

Table 1. Square sum of the factorial analysis of every variable evaluated during the experiment with coffee and the rhizobium strain

	Sum of mean squares		
Variables	Grafting	Inoculation	Grafting *Inoculation
DI (days)	3.625*	255.05*	7.092*
GRP (%)	9.240*	195.80*	9.240*
GH (cm)	18.01*	208.85*	147.588*
GSD (mm)	2.47*	24.49*	2.478*
RL (cm)	97.87*	2967.00*	196.34*
VR (ml)	0.921*	123.306*	0.078*
GLN (pair)	4.28*	65.28*	4.280*
FA (cm^2)	69796.74*	355675*	70810.5*
RDM (g)	1.086*	7.469*	0.919*
LDM (g)	1.557*	14.957*	1.271*

DI: Days to inosculation, GIP: Grafting inosculation percentage, GH: Graft height, GSD: Graft stem diameter, RL: Root length, GLN: Graft leaf number, RV: Root volume, FA: Foliar area, RDM: Root dry mass, LDM: Leaf dry mass, (*) significant ($p \leq 0.05$), according to the Factorial ANOVA.

Figure 1 shows the results from the interaction for the DI variable. The addition of the rhizobium strain Rpr2 favored the grafting inosculation significantly ($p<0.05$), with two-four days before the controls (absence of rhizobium inoculation). The lowest inosculation time reduction was observed for the Isla 6-14/Robusta addition (Fig. 1).

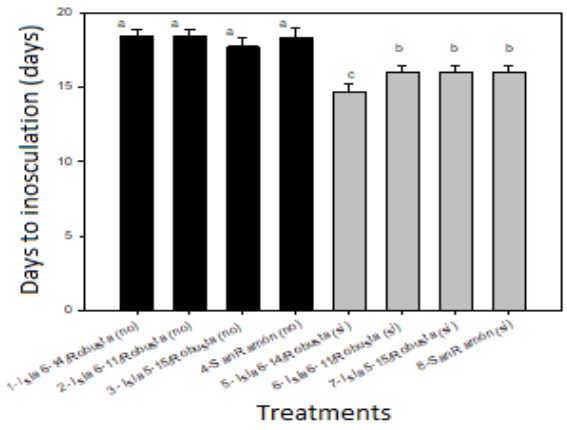

Fig. 1. Days to inosculation of *C.* arabica grafted onto *C.* canephora at 210 days. The black and gray bars show the absence and presence of rhizobium, respectively. Treatments with different scripts differ from each other, according to Tukey's test $p<0.05$, n=20

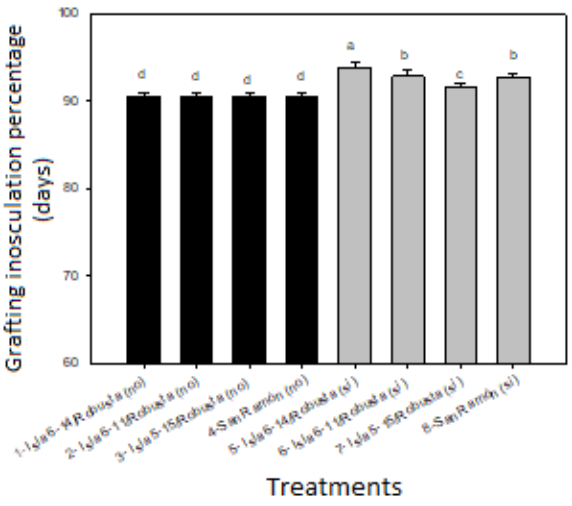

Fig. 2. Percentage of inosculation of *C. arabica* grafted onto *C. canephora* F at 120 days. The black and gray bars show the absence and presence of rhizobium, respectively. Treatments with different scripts differ from each other, according to Tukey's test $p<0.05$, n=20

A comparison of GIP showed the scion onto the Robusta stock that received the rhizobium strain Rpr2 or not (Fig. 1). The lowest values corresponded to the control grafts (90.5%), different from the grafts that received the rhizobium (statistically higher), with significant differences ($p<0.05$) from each other. The Isla 6-14/Robusta grafting reached 93.85% inosculation, higher than the ones observed in Isla 6-11/Robusta (92.8%), San Ramón/Robusta (92.7%), followed by Isla 6-15 (91.5%). These results evidenced that the inoculations with the rhizobium favored the graft inosculation percentage in the cultivars studied.

The GH, GD, GLN, LA, and LDM showed the grafting vegetative development, and had a positive effect of rhizobium application on the coffee plant

(Table 2). The extent of this effect may vary according to the combination of the grafting studied, though it can be said that the absence of rhizobium did not favor any of the variables in Table 2, ranking the lowest.

Table 2. Mean values of the evaluations of the aerial parts of *C. arabica* grafted onto *C. canephora*, in the presence or not of the rhizobium

Treatments	GH (cm)	GD (mm)	GLN (pair)	FA (cm^2)	LDM (g)
1- Isla 6-14/Robusta[1]	24.77 d	3.98 e	5.72 d	293.26 e	1.82 ef
2- Isla 6-11/Robusta[1]	24.77 d	3.98 e	5.72 d	293.58 e	1.72 f
3- Isla 5-15/Robusta[1]	24.77 d	3.98 e	5.72 d	293.93 e	1.81 ef
4-San Ramón/Robusta[1]	24.77 d	3.98 e	5.72 d	292.67 e	1.90 de
5- Isla 6-14/Robusta[2]	28.85 a	5.32 a	7.67 a	694.04 a	2.90 a
6- Isla 6-11/Robusta[2]	26.94 b	4.97 b	7.09 b	583.08 c	2.31 c
7-Isla 5-15/Robusta[2]	25.6 c	4.16 d	6.10 c	489.34 d	2.02 d
8-San Ramón/Robusta[2]	26.83 b	4.6 c	7.13 b	599.76 b	2.48 b
VC (%)	2.11	0.54	0.85	15.82	0.411

[1]not inoculated, [2] inoculated GH: Grafting height, GD: Grafting diameter, GLN: Graft leaf number, FA: Foliar area, LDM: Leaf dry mass VC: variation coefficient. Different scripts in the column mean significant differences (Tukey $p<0.05$, n=20).

For all the variables in Table 2, and according to the results of the study, the Isla 6-14 grafting onto Robusta and inoculated with the rhizobium, was statistically higher through the Tukey's test ($p<0.05$) compared to all other grafts evaluated (either inoculated or not), since they showed the greatest GH (28.85 cm), GD (5.32 mm), GLN (7.67 pairs), FA (694.04 cm^2), and LDM (2.90 g) values.

All the variables in the study showed variation coefficients that indicate the accuracy of the data collected. They were suitable experiments under controlled conditions (Table 2 and Table 3), with values below 15%, which demonstrated that the technology used was appropriate and produced reliable results.

Variables length (RL), volume (RV), and root dry mass (RDM), stood out for Isla 6-14/Robusta once more, with significantly greater development (RL 35.1 cm), and root growth (RV 6.07 mL), followed by Isla 6-11/Robusta and San Ramon/Robusta, which did not differ from each other in terms of VR ($p<0.05$) (Table 4). Moreover, the least developed roots were produced in the grafts with no inoculation and showed the lowest values. The same behavior was confirmed in all the variables, evidencing the beneficial effect of the rhizobium on coffee grafting under experimental conditions.

Table 3. Mean values of the evaluations of roots of *C. arabica* grafted onto *C. canephora*, in the presence or not of the rhizobium

Treatments	RL (cm)	RV (ml)	RDM (g)
1- Isla 6-14/Robusta[1]	20.85 d	4.44 c	5.72 d
2- Isla 6-11/Robusta[1]	21.77 d	4.09 d	5.72 d
3- Isla 5-15/Robusta[1]	22.45d	4.05 d	5.72 d
4-San Ramón/Robusta[1]	21.75 d	4.04 d	5.72 d
5- Isla 6-14/Robusta[2]	35.1 a	6.07 a	7.67 a
6- Isla 6-11/Robusta[2]	29.45 b	5.88 ab	7.09 b
7- Isla 5-15/Robusta[2]	25.95c	5.80 b	6.100 c
8-San Ramón/Robusta[2]	30.15 b	5.88 ab	7.13 b
VC (%)	1.02	0.47	0.3

[1]not inoculated, [2]inoculated; LR: root length, VR: Root volume, RDM: Root dry mass. VC: variation coefficient. Different scripts in the column mean significant differences (Tukey $p<0.05$, n=20)

The correlations obtained among the variables were used to construct a network of correlations shown in Fig. 3. It evidenced the existence of strong positive correlations (green lines), and negative correlations (red lines) among the variables. The high positive correlations (> 0.90) of the FA with RDM, RL, RV, RDM, GH, and GLN stood out. There were also high negative correlations (> 0.90) of DI with RV and RDM. These results showed the existence of a balance between the development of the vegetative part and the root of the scions when characterizing these variables (Fig. 3).

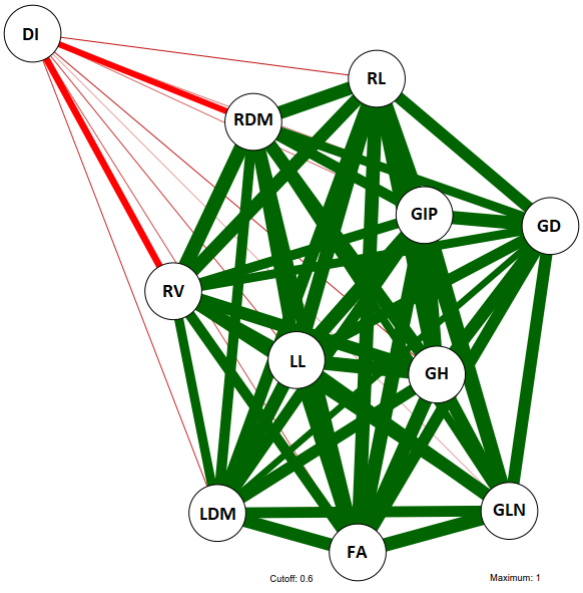

Fig. 3. Correlations network of the variables evaluated during the experiment with *C. arabica* plants grafted onto *C. canephora*, in the presence or not of the rhizobium DI: Days to inosculation, GIP: Grafting inosculation percentage, GH: Grafting height, GD: Grafting stem diameter, RL: Root length, GLN: Graft leaf number, RV: Root volume, FA: Foliar area, RDM: Root dry mass, LDM: Leaf dry mass

Discussion

The results of this research showed the positive effect of inoculating strain *R. alamii* Rpr2 on the scions, with the best responses in Isla 6-14/Robusta. Strain Rpr2 has previously demonstrated its capacity of supplying indoleacetic acid (IAA) (Hernández & Nápoles, 2019), one of the most common features of rhizobia, directly influencing plant growth (Hernández & Nápoles, 2017). IAA is involved in plant growth and development, mainly in a series of physiological processes that include cellular lengthening and division, tissue differentiation, phototropism, gravitropism, and defensive responses, and play a critical role in the formation of xylem and roots (Vega-Celedón et al., 2016), thus favoring grafting survival. Hence, the stimulation observed for all the variables in this experiment for the bacterium-inoculated grafting compared to its absence (controls), corroborated and evidenced the relevance of these rhizobia in coffee plantations.

The close physiological relationship among the variables studied in the inoculated treatment hints that Isla 6-14/Robusta showed the best root volume, which accordingly, permitted better root growth and greater rootstock and scion development, as shown by variables GH, GD, GLN, FA, and LDM. Improvements in root architecture were one of the benefits acquired from the rhizobacteria-plant interactions, as in the case of *Rhizobium*, one of the most widely known examples of such benefits (Stringlis et al., 2018). This aspect was demonstrated by Cisneros-Rojas et al. (2017) using phosphate-solubilizing bacteria on coffee plantlets.

A variation of the beneficial effect of the rhizobacterium was produced, and it was similar to the one described by Julca et al. (2018) when studying the behavior of *Coffea arabica* L. grafted onto *C. canephora* in the presence of nematodes in the nursery, thus evidencing that the responses have a genetic component manifested in the variation that can be obtained by evaluating individuals of the same plant species.

González et al. (2015) referred to the existence of a stimulus in variables height, stem diameter, leaf pairs, and dry mass of coffee trees, upon a seven-month evaluation, caused by the presence of *Rhizobium* spp., which promotes plantlet growth, as described in this experiment, with the corresponding greater development. Similar observations were published recently by Nápoles et al. (2021) when using bacterial strains identified as rhizobia, in coffee plantlets in nursery conditions, and reported improvements in the new plantlets generated through the same inoculation process.

Cupull et al. (2009) evaluated the response of the application of *Azotobacter chroococcum* on the development of hypocotyl grafting in coffee. The rootstock used was Robusta, and the scion was Isla 6-13. The results of the study indicated that the bacterium favored the scions morphologically, but with lower values than the ones described in this paper.

Barbosa et al. (2014) mentioned that the communication between the rootstock and the bud (grafting) was affected by external factors, such as the presence of nematodes, which might favor or hinder the scion growth depending on its quality and the scions used. Consequently, it can be inferred that the Isla 6-14/Robusta grafting was the most favored by the presence of the rhizobium, enabling the communication between the bud and the rootstock. Accordingly, the grafting was higher, with more leaves permitting capturing of more light and increasing photosynthesis to produce a bigger plant.

Conclusions

The inoculation of different grafting by dipping in strain *Rhizobium alamii* Rpr2 favored all the variables studied, especially genotype Isla 6-14 grafted onto *C.* canephora as a stock, Robusta.

Author contribution statement

Sucleidi Nápoles Vinent: Design of the research protocol, implementation of research activities, interpretation of the statistical results, redaction of the manuscript.

Livan González Cobas: Evaluation and compilation of the data gathered. Responsible for the validation and verification of the general replication of the experiment, and other results of this research.

Norlys Roldán Felipe: research activities and data collection.

Jorge González Aguilera: redaction, creation, and final submission of the manuscript.

Conflict of interest statement

The authors declare the existence of no conflicts of interests.

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