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Growth of Four Varieties of *Coffea arabica* L. Biofertilized with *Rhizophagus intraradices* and *Azospirillum brasilense* in Nursery

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Abstract

Coffee production has decreased due to environmental and management factors. The current plantations are old and unproductive, also due to the rust problem, caused by the fungus *Hemileia vastatrix* Berk & Br. Furthermore, the shade in the production systems has decreased with the consequent increase in soil erosion, in addition to the increase in agrochemicals. Currently, the planting of new varieties with resistance to the fungus is increasing. Furthermore, it has been shown that various biofertilized perennial crops in nursery favor their growth. In this study, the effect of applying two beneficial microorganisms, *Rhizophagus intraradices* and/or *Azospirillum brasilense*, to the planting of four varieties of *Coffea arabica* L. was evaluated. The coffee varieties marseillase, geisha, sarchimor and costa rica 95 were established in bags with the following treatments: 1) control, 2) *R. intraradices*, 3) *A. brasilense*, 4) *R. intraradices* + *A. brasilense*. Morphological and physiological yield components were recorded 168 days after transplanting. Data was analyzed statistically and differences between treatments were compared according to Tukey ($p \leq 0.05$). The results indicate that individual or combined biofertilization of microorganisms favors dry matter allocation compared to the control and the same is differentially assigned to the stem and root. The Specific Leaf Area (SLA) also showed differential response between applications of the microorganisms, in two varieties it increased when they were applied alone and in the others when they were applied together.

Keywords

Rhizophagus intraradices, *Azospirillum brasilense*, Coffee Varieties

1. Introduction

Coffea arabica L. is a strategic crop in Mexico because of its social and economic impact, and the varieties Typica, Bourbon, Red and yellow Caturra, Mundo Novo and Garnica [1] are traditionally grown in association with various timber species such as *Cedrella odorata* L. *Tabebuia donnell-smithii* Rose, or shade species, *Inga micheliana* Harms and *Cordia alliodora* (Ruiz & Pav.) Oken [2] through agrochemical-based management. In general, established varieties are old and susceptible to rust *Hemileia vastatrix* Berk. & Broome and this situation has increased the planting of new varieties with resistance to this fungus, such as Sarchimores, Geisha, Castillo, Marseillaise and Costa Rica 95 [3], which have been developed in the tropical region of America. In addition to the above, it has been demonstrated that in the initial stage of reproduction of *Coffea arabica* L., some microbiological resources of the soil favor its growth and reduce the time it remains in the nursery. Endomycorrhizal fungi have been used in *Coffea arabica* L. var Oro azteca [4], *C. canephora* (P.) ex Froehner [5], *Theobroma cacao* [6], and also in co-inoculation with bacteria such as *Azospirillum* [7] [8] and that association induces a positive effect on plant growth through the hyphae, can favor water uptake and nutrient transport to the plant, especially Phosphorus and the host plant provides carbons to the fungus [9]. This association allows reducing the use of synthetic chemical fertilizers and consequently contributes to mitigate environmental problems; in addition to favoring the increase of sustainable agriculture, food security is guaranteed and microbial diversity is maintained in the soil [10] [11]. The effects of microbial symbiosis and the host plant are generally expressed, with an increase in aerial and root biomass production [12], flowering [13], and yield. Biofertilizers are products based on a non-pathogenic microorganism that by inoculation can live associated or in symbiosis with the plant. These types of microorganisms help increase the supply, availability and physical accessibility of nutrients through various mechanisms of action and induce greater growth in the host plant [14]. However, in some cases these attributes are not expressed due to adverse effects of climatic conditions [15], in sustainable or low-input production systems, but their effective incidence depends also in the environmental and edaphic conditions, of the microorganism [16]. There is evidence that some plant associations with endomycorrhizal fungi show ecological specificity [17], and host preference [18], either with different varieties of *C. arabica* L. [19] or with mycorrhizal fungi applied to *C. canephora* (P.) ex Froehner [20]. With this background, the objective was to identify the influence of biofertilizing *Rhizophagus intraradices* and/or *Azospirillum brasilense* on the growth of four varieties of *Coffea arabica* L. at the nursery stage.

2. Materials and Methods

2.1. Location of Study Area

The research was carried out in the 2021/2022 agricultural cycle at the "San José" farm (latitude 15˚46'39"N, longitude 92˚59'49"W altitude of 1100 m above sea level) La Concordia, Chiapas, México.

2.2. Biological Material

The seeds of the Marseillaise, Geisha, Sarchimor and Costa Rica-95 varieties were obtained from seedling certified by the National Seed Inspection and Certification System (SNICS) in the state of Veracruz, Mexico, and germinated in washed and solarized river sand for four days. The endomycorrhizal fungus *Rhizophagus intraradices* (Schenk et Sm) Walker et Schuessler, was reproduced in sterile soil in the root system of *Brachiaria decumbens* Stapf. At the time of packaging there were 40 spores per gram of soil plus propagules and the level of colonization in the root system of 95% (INIFAP^{MR} Data indicated on the product). *Azospirillum brasilense* Tarrand, Krieg et Döbereiner, was produced by the company Biofabrica Siglo XXI in Xochitepec, Morelos, México, under the trade name AzoFer Plus, having a concentration of 500×10^6 bacteria·g^{-1} (Data indicated on the product).

2.3. Edaphoclimatic Conditions and Seed Germination

In "San José" orchard there is a warm sub-humid climate with rains in summer with 2000 mm of precipitation and an average temperature of 24˚C [21]. The soil belongs to the Fluvisol group and the substrate was made with the soil plus 50% washed river sand and it was solarized for 72 h with the following physical-chemical characteristics: Loam texture, 13% clay, 36% de silt and 51% sand (Bayoucus), 5.7% organic matter (Walkley-Black), 0.006 dsm^{-1} a 25˚C electric conductivity, pH 5.4 (1:2 H_2O), N total (%) 0.24, P 27.3 (mg·kg^{-1} Olsen), Fe^{2+} 26.6 (mg·kg^{-1}), Mn^{2+} 8.6 (mg·kg^{-1}), Zn^{2+} 1.5 (mg·kg^{-1}), K^+ int. 54.41 (mg·kg^{-1}), Ca^{2+} 14.9 (cmol·kg^{-1}) y Mg^{2+} 2.1 (cmol·kg^{-1}), Cu 1.04 (mg·kg^{-1}), S-SO_4 31.5 (mg·kg^{-1}) y CIC 16.5 (cmol·Kg^{-1}).

2.4. Seed Biofertilization and Experiment Setup

The seeds of the varieties for germination were moistened with carboxymethyl cellulose and impregnated with the respective biofertilizer at 4% of the weight of the seed in styrofoam trays with daily irrigation. After 48 days, more than 90% presented cotyledon leaves or "butterfly stage" and were transplanted into 10 × 20 cm black polyethylene bags. At this time, 2.5 g of each biofertilizer were added to the bottom of the hole. Four treatments were applied for each variety of coffee, 1) Control, 2) *Rhizophagus intraradices*, 3) *Azospirillum brasilense* and 4) *R. intraradices* + *A. brasilense*. In each treatment there were five repetitions and they were distributed in a completely randomized design.

2.5. Morphological and Physiological Variables

The morphological variables, plant height and number of leaves, and the physiological components were recorded (leaf area, dry biomass of leaf, stem and root) at 168 days after sowing (das).The physiological components of the yield of the aerial and root part were weighed in a semi-analytical balance (Ohaus Adventurer Pro, USA) after being dried in a forced-air oven at 60˚C - 75˚C to constant weight. Leaf area (cm^2) was obtained using a leaf area integrator (LI-COR, LI 3000[a], USA). Colonization percentage was quantified using the [22] technique. One hundred root segments 1.5 - 1.6 cm long were observed with an optical microscope with an oil immersion lens (100×).

2.6. Statistical Analysis

A completely randomized design was used, performing an ANOVA analysis of variance, using the SAS System for Windows Ver. 8.1 (1999-2000) [23], When the ANOVA was significant, the parameters were compared by Tukey test ($p \le 0.05$) and the data were plotted using Sigma Plot version 11.0.

3. Results

3.1. Morphological Components

The average height of plants in interaction with the microorganisms, alone or combined, increased 34% more, compared to the control in all varieties ($p \le 0.05$) (**Table 1**).

The tallest plants were the varieties Marseillaise, Geisha and Costa Rica 95 in interaction with *A. brasilense* biofertilization and when applied together with *R.*

Table 1. Morphological and physiological yield components of four varieties of *Coffea arabica* L. biofertilized with *R. intraradices* and/or *A. brasilense* in the nursery.

	Control	*R. intraradices*	*A. brasilense*	*R. intraradices + A. brasilense*	CV (%)
Height ($cm \cdot plant^{-1}$)					
Marseillaise	$26.8 \pm 1.0^{*c}$	41.0 ± 0.8^{b}	43.9 ± 0.6^{a}	39.1 ± 1.1^{b}	4.9
Geisha	33.1 ± 0.8^{b}	37.6 ± 0.4^{ab}	41.1 ± 1.9^{a}	43.3 ± 1.8^{a}	7.3
Sarchimor	28.4 ± 1.2^{b}	39.7 ± 0.9^{a}	38.8 ± 0.9^{a}	40.5 ± 1.0^{a}	5.7
Costa Rica 95	34.1 ± 0.7^{c}	43.2 ± 0.7^{ab}	40.7 ± 1.7^{b}	46.2 ± 0.9^{a}	5.4
Number of leaves$\cdot plant^{-1}$					
Marseillaise	6.2 ± 0.7^{b}	9.5 ± 0.2^{a}	7.7 ± 0.4^{ab}	8.2 ± 0.4^{ab}	13.0
Geisha	6.5 ± 0.2^{b}	8.2 ± 0.2^{ab}	8.0 ± 0.5^{ab}	8.5 ± 0.6^{a}	12.1
Sarchimor	7.2 ± 0.4^{a}	7.2 ± 0.6^{a}	8.0 ± 0.4^{a}	7.2 ± 0.4^{a}	13.5
Costa Rica 95	8.2 ± 0.2^{c}	10.2 ± 0.4^{a}	8.7 ± 0.4^{bc}	10.7 ± 0.4^{a}	9.1

*Values with the same letter within each factor and column are equal according to Tukey's test at $p \le 0.05$. CV = coefficient of variation (%).

intraradices. In the case of Sarchimor, this variable was also expressed with biofertilization alone with *R. intraradices*. The most notable increase in the number of leaves was in the treatments biofertilized with *R. intraradices* and *A. brasilense* alone or combined, and the lowest number in the control, except with the Sarchimor variety, which showed no difference in any treatment.

3.2. Physiological Components

A significant statistical difference ($p \leq 0.05$) was found in the biomass allocation of the different yield components of the varieties with the microorganisms. The average leaf area of the four varieties biofertilized with *R. intraradices* increased 64.2% in relation to the control. In the case of *A. brasilense* the increase represented 57% and the highest value (66.5%) was reached when the two microorganisms were applied together (Table 2).

Table 2. Physiological components of the yield of four varieties of *Coffea arabica* L. biofertilized with *R. intraradices* and/or *A. brasilense* in nursery.

	Control	*R intraradices*	*A. brasilense*	*R. intraradices + A. brasilense*	CV (%)
Leaf area ($cm^2 \cdot plant^{-1}$)					
Marseillaise	69.2 ± 0.7^c	288.0 ± 17.4^{ab}	244.5 ± 5.7^b	295.8 ± 10.0^a	9.4
Geisha	70.9 ± 0.2^c	245.5 ± 0.2^a	201.4 ± 0.5^b	258.0 ± 0.6^a	6.8
Sarchimor	107.5 ± 3.0^b	251.0 ± 7.2^a	243.8 ± 5.4^a	259.4 ± 12.7^a	9.4
Costa Rica 95	154.8 ± 5.0^d	339.4 ± 20.1^b	244.8 ± 2.9^c	390.1 ± 9.9^a	8.2
Leaf dry weight ($g \cdot plant^{-1}$)					
Marseillaise	0.40 ± 0.0009^c	1.37 ± 0.04^a	1.14 ± 0.16^b	1.10 ± 0.06^b	8.7
Geisha	0.55 ± 0.02^b	1.25 ± 0.04^a	1.12 ± 0.07^a	1.17 ± 0.06^a	10.6
Sarchimor	0.42 ± 0.01^b	0.82 ± 0.06^a	0.86 ± 0.03^a	0.98 ± 0.03^a	10.9
Costa Rica 95	0.50 ± 0.16^c	1.13 ± 0.11^{ab}	0.86 ± 0.04^b	1.46 ± 0.13^a	17.0
Stem dry weight ($g \cdot plant^{-1}$)					
Marseillaise	0.49 ± 0.03^b	0.62 ± 0.04^a	0.70 ± 0.02^a	0.59 ± 0.03^{ab}	9.4
Geisha	0.24 ± 0.02^c	0.59 ± 0.01^b	0.75 ± 0.03^a	0.55 ± 0.02^b	9.8
Sarchimor	0.14 ± 0.003^b	0.35 ± 0.01^a	0.41 ± 0.02^a	0.38 ± 0.007^a	9.0
Costa Rica 95	0.55 ± 0.03^b	0.70 ± 0.02^a	0.40 ± 0.01^c	0.59 ± 0.01^b	7.7
Root dry weight ($g \cdot plant^{-1}$)					
Marsellesa	0.18 ± 0.01^c	0.83 ± 0.02^b	1.35 ± 0.02^a	0.88 ± 0.02^b	6.2
Geisha	0.31 ± 0.01^d	0.50 ± 0.01^c	0.61 ± 0.009^b	0.72 ± 0.02^a	6.7
Sarchimor	0.21 ± 0.006^d	0.50 ± 0.01^a	0.37 ± 0.01^c	0.44 ± 0.01^b	6.0
Costa Rica 95	0.39 ± 0.008^b	0.65 ± 0.02^a	0.46 ± 0.02^b	0.61 ± 0.02^a	7.5

*Values with the same letter within each factor and column are equal according to Tukey's test at ($p \leq 0.05$). CV = coefficient of variation (%).

Among varieties, leaf area increased in Geisha and Sarchimor with *R. intraradices*, but the most generalized response was with the interaction of the biofertilization of the two microorganisms. In contrast, the Sarchimor variety showed a higher increase than the control when the microorganisms were included alone or in combination. The dry biomass of the leaf lamina was higher when the varieties were biofertilized with *R. intraradices* alone (59.6%) and when included together with *A. brasilense* (60.6%) compared to the control. Biofertilization with *A. brasilense* alone induced a slight decrease (53.5%) in leaf lamina biomass. On the other hand, the interaction between the microorganisms and the varieties showed contrasting changes. The Marseillaise variety showed an increase in leaf biomass with *R. intraradices* and Geisha and Sarchimor showed no statistical difference between the microorganisms, but they showed changes compared with the control. Among varieties, the allocation of biomass to the leaf was lowest in the variety Marseillaise (41.5%) and highest in Sarchimor (52.7%). The differences in the percentage of dry biomass allocation to the stem between the varieties with the microorganisms, alone or combined, did not present contrasting changes, that is, with *R. intraradices* and *A. brasilense*, the average was 37.5% in relation to the control, and when both microorganisms were applied together it was 32%. The average allocation of dry biomass to the stem of the varieties Marseillaise, Geisha and Costa Rica 95 represented 25.8%, and the lowest allocation of 21.7% was with Sarchimor. Root biomass in the varieties in interaction with the microorganisms increased on average 55% in relation to the control, and the highest average (60%) was when only *A. brasilense* was applied. Among varieties, Marseillaise induced greater root growth with *A. brasilense*, while Sarchimor and Costa Rica 95 did so with *R. intraradices*. In the case of Geisha, the highest value was presented with the co-inoculation of the two microorganisms. The aerial and root growth of *Coffea arabica* L. varieties showed significant variations between microorganisms and varieties (**Figure 1**). In all cases the aerial and root growth of the microorganisms in the varieties exceeded the control ($p \leq 0.05$).

The stem/root ratio presented the most contrasting changes with the Marseillaise variety in interaction with the microorganisms alone or in combination. There was a notable increase in root biomass with *A. brasilense* biofertilization, and the highest value in the stem was found when *R. intraradices* was applied. Geisha showed greater root growth with the microorganisms compared to the control, and in the stem, the biomass allocation was similar. The Sarchimor variety showed a slight increase in root growth when *R. intraradices* was biofertilized alone, and the greatest allocation of aerial biomass occurred with the double symbiosis. In contrast, the variety Costa Rica 95 induced similar root biomass allocation with and without the microorganisms and with greater induction of aerial growth when *R. intraradices* and the two microorganisms were applied together. When considering the specific leaf area (SLA) as a functional characteristic to induce growth in the varieties with the interaction of microorganisms, the highest values were found with the biofertilization of microorganisms alone

(Figure 2). In the biofertilized treatments, SLA increased compared to the control.

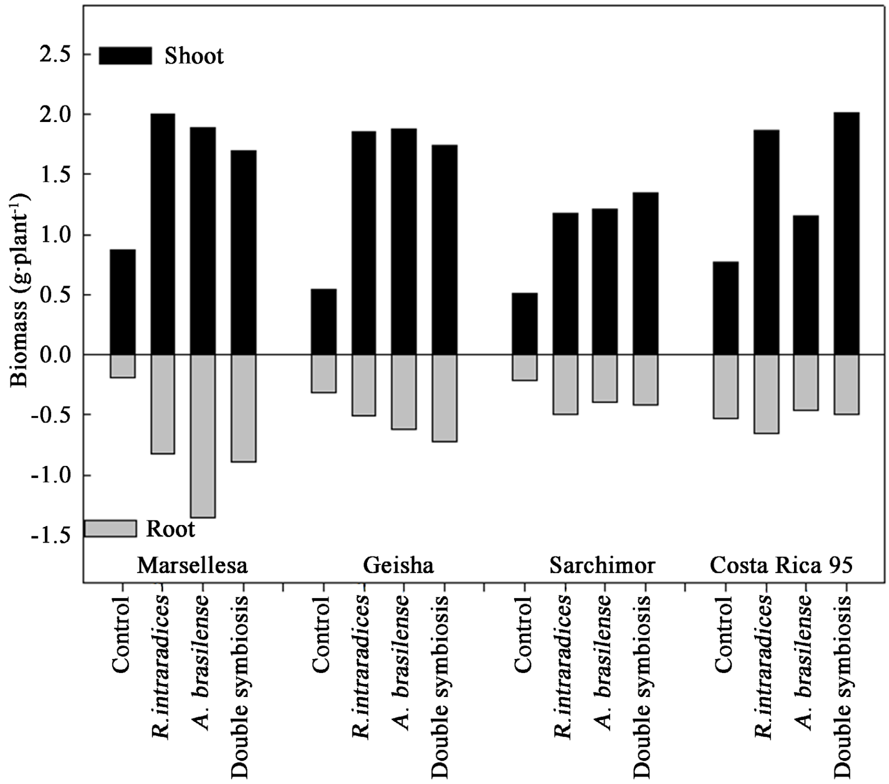

Figure 1. Root/shoot ratio of four varieties of *Coffea arabica* L. biofertilized with *R. intraradices* and/or *A. brasilense* in the nursery. The values with five repetitions averages.

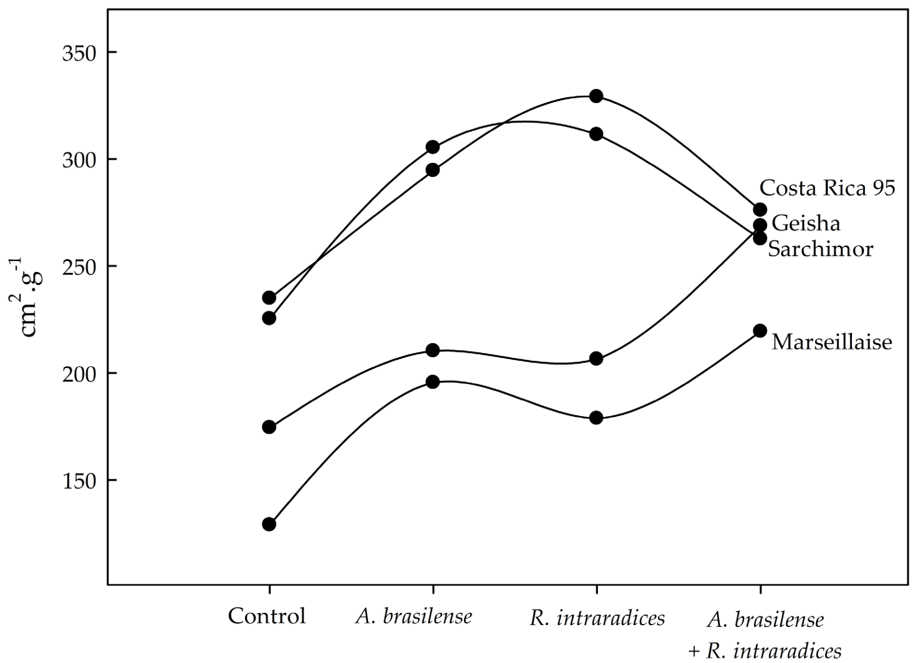

Figure 2. Specific leaf area of four varieties of *Coffea arabica* L. biofertilized with *R. intraradices* and/or *A. brasilense* in a nursery. Values with averages of five replicates.

The Sarchimor and Costa Rica 95 varieties, showed higher SLA compared to Sarchimor and Marseillaise. The previous response suggests a greater capacity to acquire resources. When correlating the root colonization of *R. intraradices* and that of the native endomicorrhizal fungi present in the substrate with the SLA, greater growth was demonstrated in plants where *R. intraradices* and *A. brasilense* were included alone or in combination (**Figure 3**).

4. Discussion

In the morphological components of yield, the increase in height of biofertilized plants reflects the benefits of the symbiosis of coffee varieties with microorganisms. However, it was different among varieties, but with greater induction when including *A. brasilense* and the double symbiosis *R. intraradices* plus *A. brasilense*. Other authors cite the increase in height in varieties of *Coffea arabica* L. var Garnica [24] and *C. arabica* L. cv. Caturra [25] with different native endomycorrhizal fungi. The co-inoculation benefits of *R intraradices* and *A. brasilense* have also been cited with height increasing capacity in *Theobroma cacao* L. [6] and in maize plants with other microorganisms besides *Azospirillum*, such as *Trichoderma*, *Pseudomonas* and the endomycorrhizal fungi *Glomus mosseae* and *G. deserticola* [26]. The differential response in plant height among varieties is attributed to intrinsic factors, such as the abundance and length of root hairs that in general determine nutrient and water uptake from the soil, and consequently can be expressed in increased growth. Endomycorrhizal fungi favor nutrient acquisition through hyphae [27] and *Azospirillum* promotes root growth

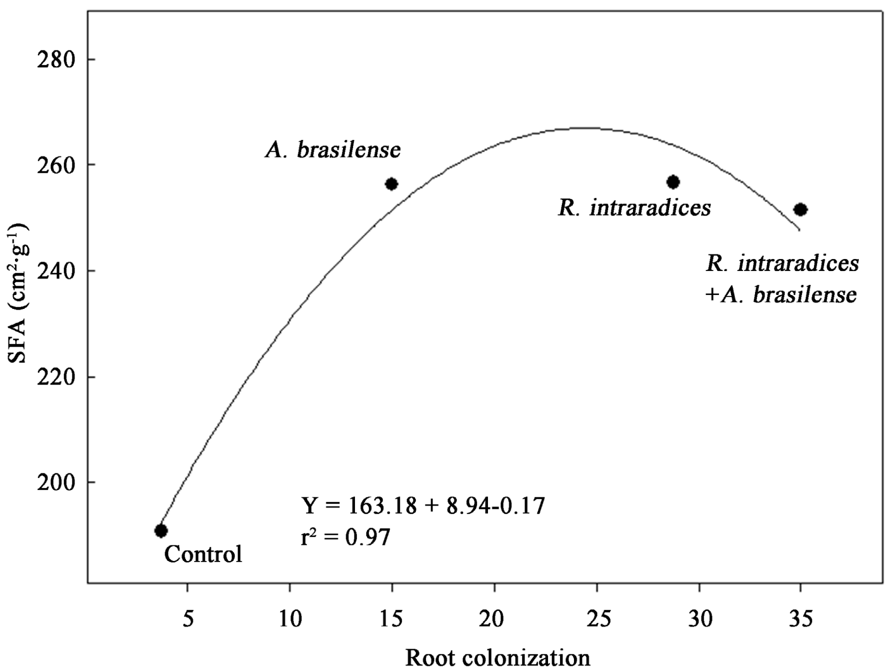

Figure 3. Relationship between root colonization and specific leaf area of four varieties of *Coffea arabica* L. biofertilized with *R. intraradices* and/or *A. brasilense* in the nursery. The values with five repetitions averages.

with the production of phytohormones such as auxins [28] that induce an increase in root number and favor the transport of minerals and water [29]. In contrast, in a different species *C. canephora* (Pierre) ex Froehner, the benefit of biofertilization was expressed in greater plant height with the application of *R. intraradices* and *A. brasilense* separately [5]. The increase in leaf number with biofertilization of *R. intraradices* and *A. brasilense* separately and together was present in three varieties, except Sarchimor. This same result, more number of leaves, has been cited in other perennial crops, such as *Theobroma cacao* L and *Coffea arabica* L. var Oro Azteca [30] and in the case of *Cedrela odorata* L. it increased by nine more leaves compared to the control [31]. In *C. canephora* (Pierre) ex Froehner the highest number of leaves is cited with biofertilization alone of *A. brasilense* [5]. The differential induction between coffee varieties and microorganisms could be related to the root exudates of each plant and their capacity to favor the colonization of other microorganisms that affect root morphogenetic development in the host plant [32], and consequently improve its nutrition and growth induction. In our case, the increase in biomass accumulation in the treatment with the two microorganisms together indicates their functional affinity with the plant and suggests that the host plant was able to supply sufficient carbon to the microorganisms. Of the physiological components of yield, the greater increase in leaf area when applying both microorganisms together in the coffee varieties suggests functional compatibility of the host plant with the microorganisms, even though the endomycorrhizal symbiosis lacks of taxonomic specificity [33], however, a certain preference seems to occur between the plant and the introduced microorganisms. Several studies have shown that co-inoculation of plants with fungi and bacteria induces synergistic effects in their interaction [34] [35], even though root colonization increases the demand for carbohydrates with the co-inoculation of more than one microorganism, and it has been estimated that the plant in symbiosis with endomycorrhizal fungi transfers about 20% of the total carbon assimilated [36]. In our case, the increase in biomass accumulation in the treatment with the two microorganisms together indicates their functional affinity with the plant and suggests that the host plant was able to supply sufficient carbon to the microorganisms. The benefits of mycorrhizal symbiosis in inducing greater leaf area in the host plant have also been found in species such as *Tabebuia donnell-smithii* Rose [37], *Eucalyptus camaldulensis* Dehnn [38] and *Cedrela odorata* L. [31]. The increase in differential dry matter accumulation in leaf biomass with biofertilization alone of *R. intraradices* confirms the improvement in nutrient uptake and overall plant productivity of the host plant [39]. They [40] point out the importance of mycorrhizal plants nutrition and their improvement in photosynthesis, as a response to the increase of the external hyphae that allows exploring a greater volume of soil and extends the surface area of nutrient uptake [41]. Similar results are cited [42] when evaluating different endomycorrhizal fungi in the plant development of *S. rebaudiana* Bertoni. The response of the varieties to biofertilization by separate microorganisms for biomass allocation to the stem suggests differential allocation of photosynthates, furthermore, influenced by the modification of the microbial community of the rhizosphere as a consequence of root exudates [43]. This background proposes the contrasting functionality of microorganisms in interaction with plants [44], such as the response found with *L. leucocephala* [8], that symbiosis induces changes in its physiology [45]. In *Coffea arabica* L. var Oro azteca with *R. intraradices* increases stem biomass [4]. The lower dry matter allocation to the root system in the Marseillaise and Geisha varieties with *R. intraradices* is likely due to the substitution of root hair growth by fungal hyphal growth. It appears that the fungal hypha replaces root hairs and the plant transports more photosynthates to the aerial part for biomass production. This fact could be due to less allocation of carbohydrate, a product of photosynthesis, to the elaboration and maintenance of the root system, with a consequent benefit for aerial plant growth [38]. Similar results are cited with the biofertilization of *R. intraradices* in plants of *Tabebuia donnell-smithii* [46] and *Theobroma cacao* L [6]. In contrast, with the varieties Sarchimor and Costa Rica 95, the root system increased with *R. intraradices* biofertilization compared to the other treatments. This same effect was cited by [5], in *C. canephora* with the same endomycorrhizal fungus and [4] with *C. arabica* var Oro azteca. Root growth when *A. brasilense* was biofertilized induced greater growth contrast in the root system of the Marseillaise variety. This is suggested as a response to the production of phytohormones by the bacterium [47] [48], such as indole acetic acid [47] [48] [49], cytokinins and gibberellins that induce more root hairs and consequently favor nutrient uptake [50]. In annual crops, *A. brasilense* also induces an increase in root biomass when applied in co-inoculation with endomycorrhizal fungi on *Phaseolus vulgaris* L. and *Zea mays* L. [15]. Regarding to the allocation of biomass to the stem and root, the differences indicate interaction between the plant and the microorganisms and this same effect is cited [51]. In the case of *R. intraradices*, the benefits in both aerial and root organs of plants are associated with nutrient and water transport [38]. The transport of nutrients and water to the plant increased because the hyphae are thinner than the roots and can access places where roots or root hairs do not normally penetrate, thus increasing their ability to explore a larger volume of soil for nutrient uptake [41] [52]. The increase in root/stem ratio seems to be related to the modular growth of plants. According to [53], growth is regulated by genetic traits that vary only in a specific range of phenotypic plasticity. The expression of modular growth of the plant strata, physiologically holds the modules together and integrates a whole, with a typical exponential growth phase, followed by a period in which the rate of iteration of new modules and biomass accumulation declines until the maximum size is reached [54]. In general, biofertilization of *Coffea arabica* L. with *R. intraradices* and *A. brasilense* significantly increases dry matter allocation to the different yield components, compared to the control [55] [56], and in some isolates more of them are expressed, or when biofertilizing the plant with different fungi in consortium [25]. The lower growth induction of some plants can be attributed to certain environmental and soil conditions that may influence the results of biomass induction. The endomycorrhizal fungi in one soil behave differently from those in other soils [57]. It is also highly influenced by several environmental factors including climate conditions, age and variety of host plant [58]. The specific leaf area (SLA) of the four varieties suggests differential response among varieties to the gradual increase in assimilatory tissue [59], in response to the transport of nutrients to the plant [60] due to the increase with the biofertilization of microorganisms compared to the control, but in two of them, Geisha and Marseillaise, the lowest SLA was when the microorganisms were introduced separately, whereas in the varieties Costa Rica 95 and Sarchimor the increase was greater when they were biofertilized alone. The greater increase in SLA with *R. intraradices* biofertilization on Geisha and Sarchimor suggests the ability of the endomycorrhizal fungus in transporting phosphorus to the plant [61] [62], or to a differential induction response of the microorganisms in stimulating growth of yield components, or also, between differences in the way they are exploited [54]. The presence of root colonization in the control is due to the fact that solarization does not destroy the spores contained in the substrate. The lower root colonization values in the control suggest a lack of compatibility of the native fungi with the varieties, which have different origins [63] [64] [65] even though, the symbiotic association between higher plants and mycorrhizae is commonly found in nature. In the *A. brasilense* case, root colonization by endomycorrhizal fungi was higher than the control and this effect could have been favored by root exudates. This may be due to the generation of specialized metabolites, such as flavonoids, which generate communication with other members of the phytobiome [66] such as endomycorrhizal fungi [67] and thus improve their acquisition of nutrients and water. In the case of flavonoids, they are considered signaling compounds for endomycorrhizal fungi that can influence spore germination, hyphal growth, and root colonization [68]. The lower root colonization values in the control suggest a lack of compatibility of the native fungi with the varieties, which have different origins. However, symbiotic association between higher plants and mycorrhizae is commonly found in nature.

5. Conclusion

Biofertilization of the four *C. arabica* L. varieties in nursery with some of the individually biofertilized microorganisms and in co-inoculation, favored growth and dry matter allocation of morphological and physiological yield components compared to the control without biofertilization. The most contrasting changes in dry matter allocation in the four coffee varieties occurred at 56 and 84 ddt and the highest and most recurrent plant expression arose with the biofertilization of the two microorganisms. The aerial and root biomass of the varieties of *Coffea arabica* L. expressed differential response in interaction with the microorganisms alone or in co-inoculation. The most remarkable increase of SLA is when the microorganisms are applied separately in two varieties and in the others with the co-inoculation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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