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# Response to the application of Pectimorf® and Viyoya® to coffee tree seedlings regenerated from germplasm conserved *in vitro* during the acclimatization phase

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## Abstract

The employment of biotechnological methods in conservation and multiplication of promissory genotypes of coffee is significant, but one of the limitations is the management of vitroplants during the adaptation phase. The aim of this research was to analyse the response of an F1 hybrid seedlings of coffee to the acclimatization phase with the application of new biofertilizers, respectively Pectimorf® and Viyoya®. As plant material seedlings of hybrid F1 H-434 obtained by somatic embryogenesis were used. The effect of Pectimorf® was determined in interaction with *Rhizophagus intraradices* and *Funneliformis mosseae*. In the second experiment, the growth and development of coffee seedlings was evaluated, with a biofertilizer based on efficient microorganisms (Viyoya®) combined with arbuscular mycorrhizal fungi. The study was carried out under greenhouse temperature of 25 °C and relative humidity of 75%. The results showed positive responses to the acclimatization of the seedlings with the bioproducts, in nutrition and growth. Concentrations of 10-20 mg $L^{-1}$ of Pectimorf® guaranteed significant average increases for height, stem diameter and pairs of leaves, in combination with the *R. intraradices* strain (T3, T5). In addition, it had been seen that a favourable percentage of infestation was obtained in the soil used. The treatments with 1 and 3 mL of Viyoya® in combination with the AMF strains showed an increase in the indicators of plant height, leaf pairs and stem diameter, as well as the leaf area, the values being higher when applying 3 mL of Viyoya® (T3, T4, T5, T6). These results demonstrate the usefulness of plant growth promoting products in the acclimatization phase of an F1 hybrid seedlings of coffee.

***Keywords:*** acclimatization; *Coffea arabica* L.; HMA; *in vitro* culture; Pectimorf

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## Introduction

Coffee (*Coffea arabica* L.) occupies a significant place in the international beverage market, being a fundamental element in the daily life of the most diverse populations in most of the planet, where it is enjoyed as a complement to daily activities; for this reason, it is grown with great interest in a great number of countries of Latin America, Africa, and Asia (ICO, 2019). In Latin America, it is an item of huge importance given its production and wide use. The application of somatic embryogenesis for the accelerated multiplication of coffee plants has been developed from numerous techniques that simplify this process, which seeks to establish the production of plants with great potential and the reduction of the production cost of seedlings (Rezende *et al*., 2012; Montes-de Godoy, 2019). Given the potentialities of biotechnology, it is very positively valued to take its advances to the coffee sector (De Los Santos and Hernández, 2006), since this method can contribute to an increase in yields due to the rejuvenation and sanitation of plant material.

Somatic embryogenesis, a biotechnology that allows the efficient multiplication of plants, enhances the actions of accelerated propagation of cultivars of interest and the obtaining of a high number of improved and quality materials, constituting a useful tool to speed up programs of massive propagation of plants and genetic improvement (Hazubska-Przybył *et al*., 2022). In the case of *C. arabica*, the most important species of the Coffea genus, there is special interest in hybrids and elite plants, due to their benefits for propagating genetic lines. However, it is noteworthy that the presence of hyperhydrated plants, the scarcity of studies under natural conditions, among others, are aspects that have limited its application in *in vitro* production on a commercial scale (Suárez-Castellá *et al*., 2012).

Acclimatization, defined as the environmental adaptation of plants obtained by tissue culture or *in vitro* propagation, moved to a new environment – greenhouses or field – is one of the most important phases of micropropagation (Teixeira da Silva *et al*., 2017). It is suggested that sometimes significant percentages of seedlings produced *in vitro* are lost before they can be established under field conditions (Clapa *et al*., 2013), especially since these seedlings during continuous growth and development require an adaptation process. Plants achieved *in vitro* are very sensitive to abrupt environmental changes, such as those that take place when moving to ex vitro conditions in natural environments, as well as to the influence of biotic and abiotic factors (Chandra *et al*., 2010).

The inoculation with biofertilizers produced from Arbuscular Mycorrhizal Fungi (AMF) has shown beneficial effects on the development of the coffee trees, during the acclimatization of plants obtained *in vitro* in the nursery phase (González and Rodríguez, 2004; Del Aguila *et al*., 2018), such as the increase in survival of plants at the moment of the transplant (Trejo *et al*., 2011). These endosymbiont organisms are an important part of the soil microbiome, since they facilitate the absorption of water and elements such as P, Zn, Cu, among others (de Novais *et al*., 2014; Fusconi, 2014; Sugiura *et al*., 2020; Max and Ludewig, 2021). Also, the established symbiosis with the AMF increases growth regulators, flavonoids and isoflavonoids (Salloum *et al*., 2019) that intervene in the vital functions and vigor of plants (Kiers *et al*., 2002). The coffee plants are benefited from the mycorrhizal association because the AMF protect the plant against pathogens and diseases, increases water absorption, as well as increases the adaptation of plants when transplanted from nurseries to the field (França *et al*., 2016). These fungi are important in coffee plant nutrition, nevertheless, the variety should be considered when introducing AMF, as not all associations has been efficient (Fonseca *et al*., 2019).

In recent years and because of making production systems more efficient, different laboratories and agrochemical industries have placed on the market nutritional complexes that contain micronutrients, amino acids, plant extracts, among others, which have been called "growth promoters or biostimulants". Many of these are natural biodegradable substances. Among these is Pectimorf®, a Cuban bioregulator obtained from residues of the citrus industry, whose active principle is a mixture of oligosaccharides of peptic origin (INCA, 2003). The ability of Pectimorf® to stimulate growth and significantly increase the development and vigor of *in vitro* plants of different crops, validate it as a promising alternative in plant biotechnology (Izquierdo *et al*., 2016; Mogena *et al*., 2018).

Similarly, in order to implement new technologies made with local resources, economically feasible and environmentally sustainable, the production of a new biofertilizer based on efficient microorganisms (EM) has been developed, named Viyoya®. This has not been used up to now in order to evaluate its potentialities in the development of coffee seedlings obtained *in vitro*, so delving into its effects is of great importance today.

For all the above, the aim of this research was to analyze the response to the phase of acclimatization of seedlings of an F1 hybrid of coffee, obtained by somatic embryogenesis starting from germplasm conserved *in vitro*, with the application of Pectimorf® and Viyoya® biofertilizers (new Cuban bioproducts) combined with two strains of mycorrhizal fungi.

## Materials and Methods

*Plant material*

The investigations were developed in the adaptation area of the Department of Genetics and Plant Improvement, of the Instituto Nacional de Ciencias Agrícolas (INCA), located in San José de Las Lajas, Mayabeque province, Cuba.

As plant material for the tests, during the acclimatization phase, 700 seedlings of the intraspecific hybrid F1 H-434 of *Coffea arabica* L. were used, obtained by somatic embryogenesis according to the methodology of González et al. (2005), from germplasm preserved in minimum conditions growth, in an *in vitro* collection of *Coffea* sp. The 700 selected seedlings were characterized by adequate health, height between 3-5 cm, 4 to 5 pairs of leaves, favorable development of the root system and excellent vigor and color.

*Experimental design and treatments*

<u>Experiment 1</u>

The effect of the combination of oligogalacturonides, with a degree of polymerization between 12 and 14 (Pectimorf®) (INCA 2003), was determined in interaction with two levels of strains of mycorrhizal fungi (AMF), on the growth and development of the seedlings of the hybrid F1 H-434 (Table 1).

**Table 1.** Characteristics of the studied treatments

| Treatments Experiment 1 | | | Treatments Experiment 2 | | |
|---|---|---|---|---|---|
| No. | Pectimorf® (mg $L^{-1}$) | Strains of AMF | No. | Viyoya® (mL) | Strains of AMF |
| 1 | 0 | *R. intraradices* | 1 | 0 | *R. intraradices* |
| 2 | 0 | *F. mosseae* | 2 | 0 | *F. mosseae* |
| 3 | 10 | *R. intraradices* | 3 | 1 | *R. intraradices* |
| 4 | 10 | *F. mosseae* | 4 | 1 | *F. mosseae* |
| 5 | 20 | *R. intraradices* | 5 | 3 | *R. intraradices* |
| 6 | 20 | *F. mosseae* | 6 | 3 | *F. mosseae* |
| 7 | 30 | *R. intraradices* | 7 | 5 | *R. intraradices* |
| 8 | 30 | *F. mosseae* | 8 | 5 | *F. mosseae* |
| 9 | 0 | - | 9 | 0 | - |
| 10 | 10 | - | 10 | 1 | - |
| 11 | 20 | - | 11 | 3 | - |
| 12 | 30 | - | 12 | 5 | - |
| 13 | - | *R. intraradices* | 13 | - | *R. intraradices* |
| 14 | - | *F. mosseae* | 14 | - | *F. mosseae* |

Four concentrations of Pectimorf® were evaluated: 0 mg $L^{-1}$ (P1), 10 mg $L^{-1}$ (P2), 20 mg $L^{-1}$ (P3) and 30 mg $L^{-1}$ (P4). The application was made through the immersion of the roots of the seedlings in the solution of the indicated concentration of the bioproduct, during an exposure period of two hours, based on previous results for this crop (data without publishing).

In the case of arbuscular mycorrhizal fungi, two strains *Rhizophagus intraradices* (Schenck & Smith) and *Funneliformis mosseae* (Nicolson & Gerddeman), both certified by the INCA Department of Biofertilizers and Plant Nutrition, were studied. The inoculum with spores, hyphae and infective roots was applied at a rate of 10 g/bag at the time of sowing, placing the small seedling on it (González and Rodríguez, 2004).

<u>Experiment 2</u>

The growth and development of coffee seedlings was evaluated, through the estimate of different indicators of the growth (plant height, stem diameter, leaf pairs), with the use of the new biofertilizer based on efficient microorganisms (EM) called Viyoya®, combined with arbuscular mycorrhizal fungi (Table 1). The study was carried out under greenhouse conditions and *in vitro* seedlings of the hybrid F1 H-434 of *C. arabica* were used as plant material, with characteristics like those used in Experiment 1.

In this case, the treatments consisted of different concentrations of the biofertilizer based on efficient microorganisms: 0 mL (ME1), 1 mL (ME2), 3 mL (ME3) and 5 mL (ME4), combined with two levels of AMF (*R. intraradices* and *F. mosseae*).

The applications were made by irrigating the biofertilizer with a manual sprayer, with an application every fifteen days under greenhouse conditions, for a period of 6 months.

<u>Generality of experiments</u>

The investigations were carried out under greenhouse conditions, covered by a plastic mesh (Saran). The seedlings were planted in black polyethylene bags, 14 cm x 20 cm, using 750 g of substrate in each bag, composed of leached Ferralitic Red soil (Eutric Rhodic Nitisol) (Hernández *et al*., 2015), and earthworm humus as organic fertilizer, in a ratio of 3:1 (v/v), without mineral fertilizers. The substrate used was characterized by its fertility, presenting pH values between acid and slightly alkaline, as well as low phosphorus content and somewhat high potassium content. The substrate was sieved and subsequently sterilized through dry heat in an oven at 200 °C for 3 hours. The indications recommended by the Technical Regulations, applied to the cultivation of coffee in traditional nurseries (MINAG, 2012), were followed.

The irrigation was carried out with micro-spray, with sprinklers of low pressure (2.0 kg $cm^{-2}$ and a flow of 122 L $h^{-1}$). During the first days of cultivation in this phase four daily waterings (8:00 am, 10:00 am, 12:00 pm and 4:00 pm) during one minute each one was carried out. On the fifth day three daily waterings were applied (8:00 am, 12:00 pm and 4:00 pm) with a duration of two minutes each one. With this frequency a relative humidity was guaranteed inside the house of cultivation at 75 - 80% (Martínez *et al*., 2019). All this considering the characteristics of the seedlings when released from *in vitro* conditions to the *ex vitro* phase, and after that period according to the requirements of the plant. During the development of the experiment, a gradual regulation of the illumination to 70% was carried out with the use of plastic mesh (Saran), which allowed the passage of only 70% of natural lighting.

In both experiments, 210 days after planting, several variables were evaluated: plant height (cm) (with a graduated ruler from the base of the stem), stem diameter (cm) (with a calibrator), number of pairs of leaves, dry mass of the aerial part and leaf area according to Martín *et al*. (2006). The index of mycorrhizal infection was also determined by the Phillips and Haymand (1970) staining method. The determination was made with the objective of characterizing plant growth and determining the mycorrhizal efficiency.

The acclimatization conditions were characterized by an environment with a predominant daytime temperature of 25±2 °C and relative humidity of 75-80%.

*Statistical analysis*

In both experiments, a randomized block factorial experimental design was applied. 24 seedlings per plot were used and eight were evaluated in each of the four replicates that were carried out during the experiments. The experimental results were subjected to a double ANOVA, applying Duncan's multiple range comparison test with $P \leq 0.05\%$ as a comparative criterion between the different treatments. Statistical data were processed by the statistical package STATISTICA (StatSoft, 2007).

## Results

*Effect of the combination of Pectimorf® in interaction with two strains of arbuscular mycorrhizal fungi on the ex-vitro development of coffee seedlings*

The results obtained showed positive responses to the acclimatization of the seedlings obtained *in vitro* from the hybrid F1 H-434 of *C. arabica*, as well as to the treatment with the bioproducts, microbial and non-microbial, in nutrition and growth at this stage of significant importance during the micropropagation process. These answers support the use of biological methods to guarantee adequate fertilization, as necessary elements to obtain a high productive quality.

The statistical analysis showed highly significant differences for the variables that were evaluated between the plants treated with oligogalacturonides and the controls. The treatment with Pectimorf® was effective on the different indicators evaluated (Figure 1). Concentrations of 10 mg $L^{-1}$ (P2) and 20 mg $L^{-1}$ (P3) guaranteed significant average increases for height, stem diameter and pairs of leaves, in combination with the *R. intraradices* strain (T3 and T5) as well as 20 mg $L^{-1}$ (T11) and *R. intraradices* (T13) without combination.

Some seedlings grown in the P4 treatment exhibited less response capacity in terms of leaf area, in the same way the height was significantly lower. In this experiment, a higher mycorrhizal efficiency was achieved with the species *R. intraradices*, since it promoted the highest values for the agronomic variables evaluated with the different concentrations of Pectimorf®, with significant increases being observed with respect to the treatment without this bioactive product. Apparently, there is greater affinity between this strain and the crop under study, which results in a greater beneficial effect on the coffee plants evaluated.

An effect dependent on the concentration of the bioproduct was observed in the growth of the seedlings since a sharp decrease in the indicators studied was observed when the assayed concentration of Pectimorf® was higher than 20 mg $L^{-1}$. This effect could be attributed to the inhibition of certain processes given the elevation of the biostimulant levels.

The effect of the treatments was reflected in the variable pairs of leaves of the coffee seedlings (Figure 1), which increased when using the concentration of 10 and 20 mg $L^{-1}$ of Pectimorf® in combination with the *R. intraradices* strain (T3 and T5) and with 20 mg $L^{-1}$ (T11) and *R. intraradices* (T13) without combination. The positive effect of Pectimorf® was also evidenced in the aerial dry mass indicator (data not shown), which in this case increased with both concentrations of the bioproduct (10 and 20 mg $L^{-1}$).

Through the results shown in Figure 1, it can be seen that a favourable percentage of infestation was obtained in the soil used, from which it is inferred that it is characterized by a high mycorrhization capacity. These data are of interest, since they provide information regarding the application of AMF in planting material obtained by biotechnological methods, and in relation to mycorrhizae, the current challenge is to achieve their integration with intensive and sustainable agricultural systems in crops of agricultural and industrial importance.

It was observed that the inoculation with the AMF strains affected the infection rates, with differences between them, although the combination with the non-microbial bioproduct shows that both had influence on the indicators evaluated and the development of the coffee seedlings of the hybrid F1 H-434 obtained *in vitro* (Figure 1). Therefore, the observed results are also attributed to the various favourable effects of AMF, including increased nutritional capacities (P, N, K, Ca, Mg and micronutrients), as well as plant tolerance to stress; they also channel the beneficial effect of other rhizospheric microorganisms.

The *F. mosseae* strain exhibited the lowest infection values (Figure 1), which suggests that it infected, but was not effective for these purposes; that can be related to the acidity of the substrate used in these investigations.

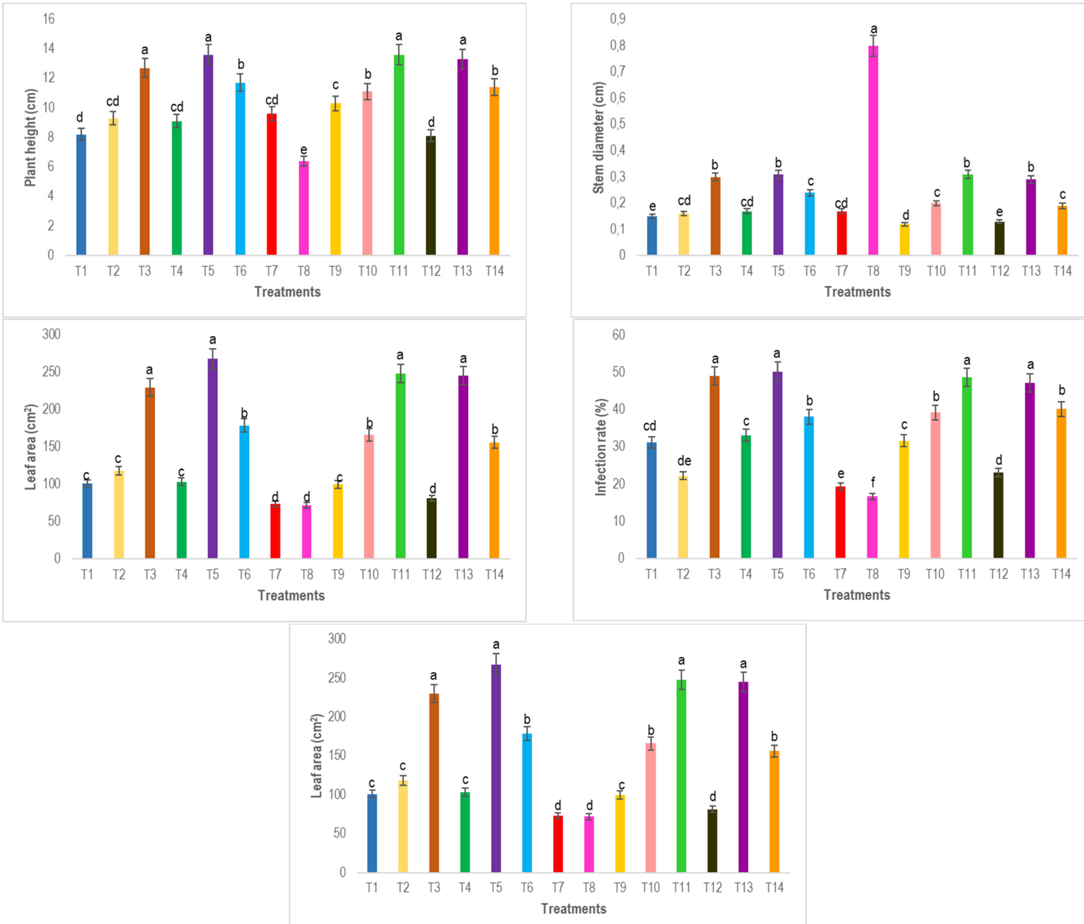


**Figure 1.** Effect of Pectimorf® and two AMF strains on the growth of seedlings of the hybrid F1 H-434 of *Coffea arabica* L., during acclimatization where: T1= P1+ *R. intraradices*; T2= P1+ *F. mosseae*; T3= P2+ *R. intraradices*; T4= P2+ *F. mosseae*; T5= P3+ *R. intraradices*; T6= P3+ *F. mosseae*; T7= P4+ *R. intraradices*; T8= P4+ *F. mosseae*; T9= P1; T10= P2; T11= P3; T12= P4; T13= *R. intraradices*; T14= *F. mosseae*

The results of the combination of Pectimorf® at 20 mg $L^{-1}$ with the two strains of AMF are showed in Figure 2.


**Figure 2.** Seedlings of the hybrid F1 H-434 of *C. arabica* under acclimatization conditions, from the treatment with bioproducts. A: Pectimorf® (20 mg $L^{-1}$) and AMF (*F. mosseae*); B: Pectimorf® (20 mg $L^{-1}$) and AMF (*R. intraradices*)

The combination of Pectimorf® (20 mg L$^{-1}$) and *R. intraradices* allowed to obtain the best results and greater efficiency in the vegetative growth of the coffee hybrid that was studied in the present investigation. These results show that not all inoculums work the same way.

In general, for the parameters analysed here the best treatment were T3 (10 mg L$^{-1}$ of Pectimorf® + *R. intraradices*), T5 (20 mg L$^{-1}$ Pectimorf® + *R. intraradices*), T11 (20 mg L$^{-1}$ of Pectimorf®) and T13 (*R. intraradices*).

*Effect of the combination of the biofertilizer Viyoya® in interaction with two strains of arbuscular mycorrhizal fungi in the ex-vitro development of coffee seedlings*

In this research, the treatments with 1 and 3 mL of Viyoya® in combination with the AMF strains *R. intraradices* (T3 and T5), 3 mL of Viyoya® (T11) and *R. intraradices* (T13) showed an increase in the indicators of plant height and leaf pairs with statistically significant differences compared to other treatments (Figure 3). Referred to stem diameter the best treatment was 5 mL of Viyoya® with *F. mosseae* (T8) followed by T3, T5, T11 and T13.

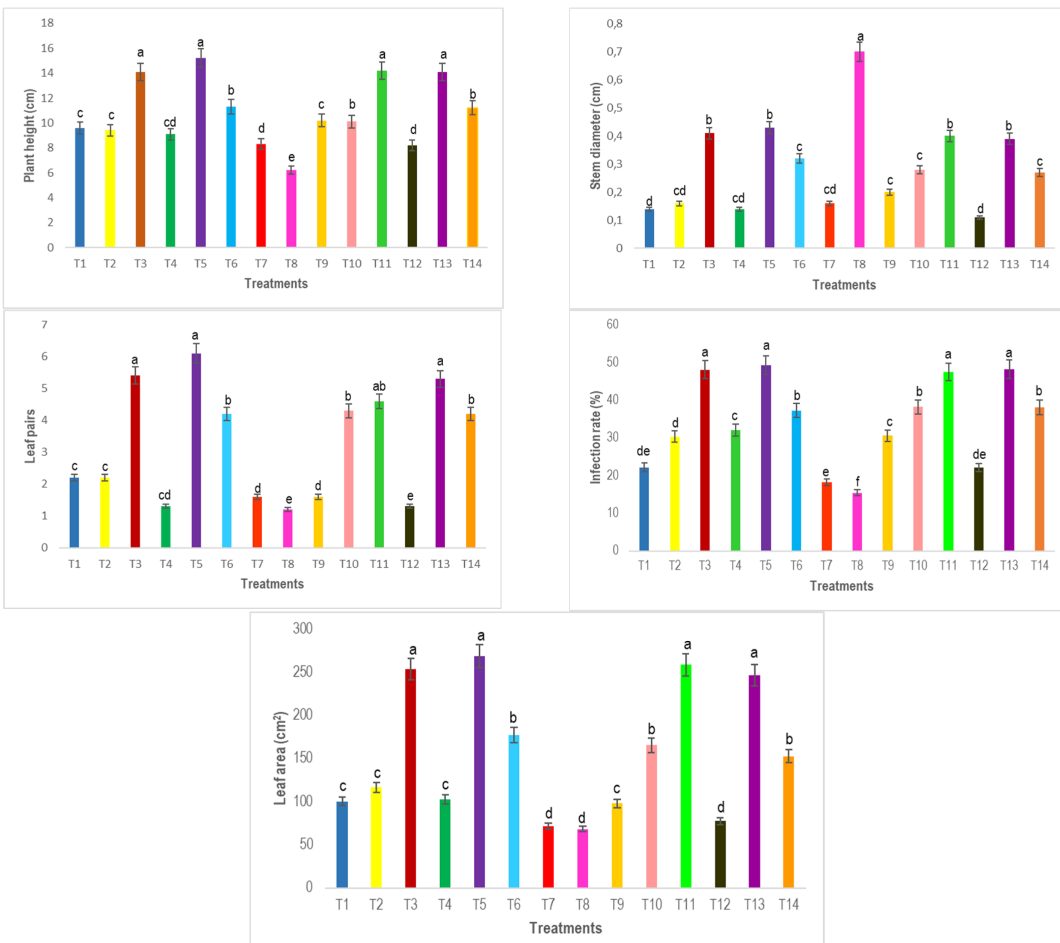


**Figure 3**. Effect of the Viyoya® biofertilizer and two AMF strains on the growth of seedlings of the hybrid F1 H-434 of *Coffea arabica* L., during acclimatization where: T1= ME1+ *R. intraradices*; T2= ME1+ *F. mosseae*; T3= ME2+ *R. intraradices*; T4= ME2+ *F. mosseae*; T5= ME3+ *R. intraradices*; T6= ME3+ *F. mosseae*; T7= ME4+ *R. intraradices*; T8= ME4+ *F. mosseae*; T9= ME1; T10= ME2; T11= ME3; T12= ME4; T13= *R. intraradices*; T14= *F. mosseae*

The leaf area also achieved increases with these treatments (T3, T5, T11 and T13) since the beginning of the study, the values being higher when applying 3 mL of Viyoya® combined with *R. intraradices* (T5). This is an indicator of interest since it is directly related to the useful photosynthetic area of the plant.

The infection rates of *R. intraradices* increased with the combination of 1 and 3 mL of Viyoya® like the experiment with Pectimorf® showing again that this bioproducts favourably affect the colonization of this fungus in the roots of the coffee plants studied.

## Discussion

The application of bioproducts and AMF strains *R. intraradices* and *F. mosseae* for the acclimatization phase of the hybrid F1 H-434 of *C. arabica* provided positive results in seedlings growth, taking in account the importance and difficulty of this stage for plants obtained by *in vitro* culture. According to Mokhtarzadeh *et al*. (2013) for acclimatisation phase, it is necessary to transfer the vitroplants to proper growing substrate so plantlets may not face difficulties and they can have a proper development and growth. In this research bioproducts and two AMF strains play the role of substrate providing to the plantlets the conditions for a better adaptation and development.

There is a history of the use of Pectimorf for biotechnological purposes, demonstrating its capacity as a substitute for auxins and cytokinins, in different stages and in several crops such as sugar cane, citrus fruits, potatoes, tomatoes, tobacco, bananas, rice, garlic, among others (Cid *et al*., 2006; Izquierdo *et al*., 2009; Nieves, 2013). Benefits to the culture have also been observed in various processes such as rooting, shoot formation and particularly in the *ex-vitro* adaptation phase (Castilla and González, 2016). In this investigation Pectimorf applied at 10 mg $L^{-1}$ (P2) and 20 mg $L^{-1}$ (P3) significantly increased height, stem diameter and pairs of leaves of seedlings when it was combined with *R. intraradices* strain.

The simple treatment with the AMF strains improved the response of the evaluated indicators, but in a very discreet way. However, the effect of the AMF-non-microbial bioproduct combination (Figure 1), although it depended on the concentration tested, showed a positive effect for the growth indicators under study. In general, a certain tendency to increase mycorrhizal infection was observed with intermediate concentration values of the bioproduct used. Similar results were obtained by Borriello *et al*. (2017) with *Ranunculus asiaticus* L. treated with *F. mosseae*, *R. intraradices* and *Glomus* sp. Also, Garmendia and Mangas (2012) point out that the use of AMF can improve platelets without fertilization.

The exogenous application of oligosaccharins influences the growth and development of plant tissues, which has been substantiated by several authors (Cutillas and Lorences, 1997; Álvarez *et al*., 2011; Corbera and Nápoles, 2013). The use of bioproducts such as these constitutes a necessity for Cuban agricultural production, since it is characterized by being ecologically balanced and economically feasible to increase the nutrition of the crop in question, its possible yields and adequate responses to effects caused by biotic stress. The oligogalacturonides that are part of Pectimorf® are extracted by enzymatic hydrolysis of citrus pectin, a raw material widely used in the food industry and the final product, once applied, is biodegradable by soil microorganisms, leaving no traces in the plant or substrate (Cabrera, 2000), which represents advantages from the environmental point of view (Nápoles-Vinent *et al*., 2017).

Generally, when *in vitro* plants are transferred to greenhouse conditions, they exhibit rapid wilting, so it is not only important to maintain a high relative humidity in the new environment, but also to be able to induce a certain level of tolerance to drought in the seedlings to preserve the mechanisms that regulate the water volume (Chintakovid *et al*., 2021) and this was achieved in this research with the application of AMF to the coffee plants. The AHM are important for the plants, since they improve the growth of the host increasing nutrient and water intake from the soil, they also protect plants from salinity, pathogens and some toxic compounds (Vaast *et al*., 2004).

In the acclimatization phase, parameters such as plant height, leaf pairs and stem diameter increased significantly with the application of 1 and 3 mL of Viyoya® (Figure 3). The results corroborate what is referred to the effect of the active substances produced by these microorganisms, which promote the growth of various crops and stimulate the plants to make use of their own resources and increase their development and growth. The seedlings, when treated with this new microbial product and the AMF strains, especially *R. intraradices*, exhibited very favourable characteristics, both from the morphological and anatomical point of view. Apparently, the mixture of substances that make up this product stimulates vegetative development. The infection rates of *R. intraradices* increased with the combination of 1 and 3 mL of Viyoya® like the experiment with Pectimorf® showing again that this bioproducts favourably affect the colonization of this fungus in the roots of the coffee plants studied.

It is known that plants grown *in vitro* are characterized by a physiology and anatomy that differ from what is observed in *ex vitro* conditions, either in greenhouses or in natural conditions (Majada *et al*., 2001). Generally, these seedlings have a poorly developed cuticle, due to the high relative humidity, the leaves are thin, soft, and photosynthetically inactive, the palisade cells, which are the ones that must use the light, are less and small. In addition, the stomata may not be sufficiently functional, and remain open when transplanted to ambient conditions, leading to water stress (Pierik, 1990). From the point of view of radical development, the emission of non-functional roots without connection with the conductive bundles constitutes one of the main disorders, all of which can affect the survival of plants in acclimatization. However, the response observed in the seedlings of the F1 H-434 hybrid of coffee studied and with the use of the different bioproduct alternatives is encouraging. Borges-García *et al*. (2015) for the acclimatization phase of 'FHIA-18' (Musa AAAB) used Pectimorf® and a significantly higher survival percentage was obtained, also a normal morphological development of the seedlings was observed. In the same way and with similar results Posada *et al*. (2016) used Pectimorf® for rooting and *in vitro* acclimatization of papaya shoot with a 76.2% survival in *ex vitro* conditions.

The experiences are not only interesting to establish the impact of this type of management, but also to improve the use of bioproducts and their employment in the adaptation of seedlings obtained by biotechnological methods.

The results obtained in this study are of great interest because although the coffee tree is a crop that can be propagated by seeds, vegetative propagation is often used to increase the number of certain select genotypes, as is the case of F1 hybrids. Also, difficulties exist in the processes of germplasm conservation of this crop due to the presence of seeds with intermediate behaviour, that is, they lose viability when they dry out below a certain moisture content. In these cases, *in vitro* conservation is a very appropriate alternative, constituting an economical, simple, and safe system that can facilitate large-scale multiplication tasks and the exchange of plant material. Providing efficient methods for the adaptation of seedlings obtained by biotechnological means from material of genotypes conserved *in vitro* thus becomes a great advantage.

## Conclusions

The beneficial effect response of microbial products and a bioactive product in the acclimatization phase of seedlings of a genotype promissory of coffee regenerated from germplasm preserved under conditions *in vitro* was demonstrated. Considering the importance of the biotechnological methods, the increase in Cuba of the demand from material for coffee plantation and the potentialities of the use of biological products for the stimulation of the crops, this may help researchers and growers looking for alternative strategies to the quick multiplication of genotypes selected by their morphological and agronomics characteristic as the F1 hybrid of coffee. These alternatives represent an advantage for nutrition and growth of the regenerated seedlings.

## Authors' Contributions

M.E.G.V, M.F.V and Y.C.V designed the study and conducted the experiments. S.P.A. and M.E.G.V analysed the data. M.E.G.V and S.P.A prepared the manuscript, E.S. and V.H.V.R. organized the data and performed the statistical analysis. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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