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Final site evaluation of Arabica coffee *(Coffea arabica* L.*)* grafted on Robusta rootstock (*Coffea canephora* P.) to the application of biostimulants.

Definitive site evaluation of Arabica coffee (*Coffea arabica* L.) grafted on Robusta rootstock (*Coffea canephora* P.) to the application of biostimulants

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Abstract: The research was carried out at the Andil experimental farm of the Universidad Estatal del Sur de Manabí, the objective was to evaluate the behavior of two genotypes of arabica coffee *(Coffea arabica* L.*)* grafted on robusta rootstock *(Coffea canephora* P.*)* to the application of biostimulants, grown in a definitive site. The research was of explanatory type, in which the experimental design of completely randomized blocks (DBCA) was applied with factorial arrangement 2^2 with adjustments of measures in time (30, 60 and 90 days), 64 plants were used, distributed in 4 treatments T1 = Sarchimor 1669 + Micorriza, T2 = Sarchimor 1669 + Trichoderma, T3 = Sarchimor 4260 + Micorriza and T4 = Sarchimor 4260 + Trichoderma, with four replications. The variables to be evaluated were: rooting, graft diameter, graft length and number of leaves in a period of 90 days. The results showed an 88% of bud break, with the exception of T1, which reached 75%. The diameter of the graft of T1 was greater with 2.4 mm, the greatest length of the graft for T2 with 3.25 cm and the greatest number of leaves for T4 with 5.00 per plant. With respect to the time factor, the analysis determined a homogeneous behavior among treatments. It was concluded that the biostimulants not used had no effect on the percentage of bud set (88%), which, although high, is not satisfactory considering that this is a definitive site crop, and it is therefore recommended that this process be carried out at the nursery stage.

Key words: Coffee genotypes**,** development, morphometrics, yield, microorganisms.

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Resumen: La investigación se realizó en la Finca experimental Andil de la Universidad Estatal del Sur de Manabí, el objetivo fue el de evaluar el comportamiento de dos genotipos de café arábiga *(Coffea arabica* L.*)* injertados en patrón robusta *(Coffea canephora* P.*)* a la aplicación de bioestimulantes, cultivados en sitio definitivo. La investigación fue de tipo explicativo, en la que se aplicó el diseño experimental de bloques completamente al azar (DBCA) con arreglo factorial 2^2 con ajustes de medidas en el tiempo (30, 60 y 90 días), se emplearon 64 plantas, distribuidas en 4 tratamientos T1 = Sarchimor 1669 + Micorriza, T2 = Sarchimor 1669 + Trichoderma, T3 = Sarchimor 4260 + Micorriza y T4 = Sarchimor 4260 + Trichoderma, con cuatro repeticiones. Las variables a evaluar fueron: prendimiento, diámetro del injerto, longitud del injerto y número de hojas en un periodo de 90 días. Los resultados determinaron un 88% de prendimiento, con excepción del T1 que alcanzó un 75%. El diámetro del injerto del T1 fue mayor con 2,4 mm, la mayor longitud del injerto para el T2 con 3,25 cm y el mayor número de hojas para el T4 con 5,00 por planta. Con respecto al factor tiempo el análisis determinó un comportamiento homogéneo entre tratamientos. Se concluye que los bioestimulantes no utilizados no incidieron en el porcentaje de prendimiento (88%) que, aunque alto, no es satisfactorio considerando que se trata de un cultivo en sitio definitivo, ante lo cual se recomienda realizar este proceso en etapa de vivero.

Palabras claves: Genotipos de café, desarrollo, morfometría, prendimiento, microorganismos.

Introduction

Coffee originated in Africa, and there are more than 100 species belonging to the *Coffea* genus. Different types of coffee plants have been developed with numerous genetic characteristics: size and shape of the plant, size and color of the fruit, resistance to diseases and pests, adaptability, productivity, etc. Two species in particular are commercially cultivated, *Coffea arabica* (arabica) and *Coffea canephora* (robusta) (Velásquez, 2019) .

Robusta coffee was cultivated mainly in Africa. This crop is considered the most resistant to changes in temperature and diseases. It is adapted to tropical climates with a rainfall between 1,000 and 1,800 mm, under a temperature of 22 and 27ºC. Its yield is much better which needs less care than arabica, one of its unpleasant

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characteristic is its bitter taste and contains 2 times more caffeine than in arabica (Pozo, 2014) .

Arabica coffee is currently grown in the mountainous areas of Chongón-Colonche, in the highlands of Olón, Pedro Pablo Gómez, Paján, Jipijapa, 24 de Mayo, Santa Ana, Pichincha, Junín, Chone, Sucre up to Jama and Pedernales in the north of Manabí. Grafting *Coffea arabica* species onto *Coffea canephora* rootstock can tolerate pest infestation, since one of the important benefits of the rootstock results in a better root system (Reyes *et al*., 2016). The *Coffea arabica* variety receives the name of arabica coffee and is considered the best quality, its production is concentrated in the provinces of Manabí (especially in the canton Jipijapa) (Venegas *et al.*, 2018).

The genotypes originating from Arabica coffee such as the hybrids Sarchimor 1669 and Sarchimor 4260, have come to gain ground in being one of the best coffee cultivars within the implementation of coffee plantations. These cultivars present excellent agronomic characteristics, guaranteeing high productivity allowing a reduction in the rate of empty fruits, they adapt very well to diverse coffee growing areas of the country, present higher production yields and to date have proven to be resistant to rust which is a plague that considerably affects coffee plantations (Parrales, 2018) .

The method of vegetative propagation by grafting is important within the coffee crop, since its main objective is to create a crop with the best attributes of two plants in one, providing it with a new root system being tolerant to biotic and abiotic factors (PDG, 2019) .

Grafting a scion of arabica coffee *(Coffea arabica L.)* onto a rootstock of robusta coffee *(Coffea canephora)* has the ability to strengthen and improve the root system, allowing the plants to be able to absorb more water and nutrients in order to generate an increase in productivity and helping arabica coffee to be resistant to phytonematodes, which are harmful, as they reduce coffee yields by damaging the roots of the plant. Due to the first species providing products of higher physical and organoleptic quality, while the second is tolerant to nematodes (Tigua, 2019) .

The queen or hypocotyledon graft, made by joining Arabica and canephora coffee, was created by the agronomist Efraín Humberto Reina in 1946. It was started by combining nematode resistant plants and high production plants**,** in this case the root and the aerial part, in order to improve the yield in production where they were affected by nematodes and minimize the use of chemical products. A rootstock is

used in the soil in this case of robusta coffee that produces stronger and deeper roots, allowing the genotype to be grafted to be provided with greater access to the passage of nutrients, water and tolerate insect pests in a better way (Castillo, 2018) .

Budding is the response manifested by a graft when the tissues of a bud adhere to the tissues of the rootstock, with nutrient exchange between them. The lack of budding can also depend on several factors, such as: poorly developed, stunted that exert a negative effect on the bud, poorly drained soils and without organic matter, diseases and pests, genetic and botanical differences in the plants to be grafted, when the cambium regions of tissues are not in close contact, inadequate temperature and humidity (Gomez, 2019) . A grafted plant could produce good quality grains, good yield of a variety, as well as resistance to pests/diseases (Fernandez, 2017).

Biostimulants They are characterized by helping plants in the absorption and utilization of nutrients, obtaining robust plants, they are also energizers of growth regulators that serve to increase yields (Torres, 2018) . They provide advantages in the stimulation and acceleration of physiological processes in cultivated plants, thanks to biostimulants the plants take ownership of nutrients, allow maximizing genetic potential, improve against abiotic stress and quality aspect in the harvest, minimizing unwanted damage to the environment (Jácome, 2015) .

Mycorrhizae define the process of symbiosis that occurs from the fungus and the roots of the plant, through the root system absorb minerals, nutrients, water found in the soil. It stimulates the rooting and growth of plants, improves the biological activity of the soil, increases the survival of root diseases and soil pathogens (Lino, 2020) . Trichoderma is a stimulator of the defense mechanism of the plants exerting protection against pathogens that cause damage to the root system and aerial part of the plant. It stimulates the growth of the crops since it possesses metabolites that initiate the processes of development in the plants, attacks pathogens of the root *(Pythium, Fusarium, Rhizoctonia)* and of the foliage *(Botrytis* and *Mildu)* and avoids the attack of *(Phytophtora)* (Chambe *et al.*, 2021).

The objective of this research is to evaluate the response of arabica coffee genotypes grafted on robusta rootstock, these species combine productivity and coffee properties that allow the level of adaptation in

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coffee growing areas, reaching as a result new varieties that are more resistant to pests and diseases

Methodology

The study was carried out at the Andil experimental farm of the Universidad Estatal del Sur de Manabí, located in the canton of Jipijapa at kilometer 5 of the road to Noboa in the canton of 24 de Mayo, in the community of Andil. Geographical coordinates between 01º 10' and 01º 47' south latitude and between 80º 25' and 80º 52' west longitude.

Jipijapa is a canton located in the province of Manabí, its limits are to the north by the cantons Montecristi, Portoviejo and Santa Ana, to the south by the province of Santa Elena and Puerto López, to the east by the cantons Paján and 24 de mayo and to the west by the Pacific Ocean. The climate of Jipijapa is tropical dry, the average temperature is 24 ° C with an average annual rainfall of 1,280 mm ², according to the Development Plan and Land Management of Jipijapa.

An experimental design of completely randomized blocks (DBCA) with a 2^2 factorial arrangement was used with adjustments of measurements over time (30, 60, 90 days). A total of 64 plants were grafted. The first factor is the varieties Sarchimor 42 60 and Sarchimor 16 69, and the second factor is the biostimulants mycorrhiza and trichodermas, which resulted in a distribution of four treatments and four replications, giving a total of 16 experimental units (EU) with six plants per EU.

To carry out the study, the following variables were taken into account: number of grafts, data were taken 30 days after grafting and expressed as percentage%; graft length, measured from graft insertion to the terminal apex of the stem in cm; graft diameter, a digital vernier caliper was used for measurements. Statistical analysis was performed using the Statistical software.

Results

The data obtained in the investigation of quantitative variables were subjected to a normality analysis in which it was determined that the data presented a normal distribution, justifying the application of

parametric statistics, analysis of variance and subsequent comparison of means.

Number of pressings

Regarding table 1, the **number of shoot emergence**, treatment three (Sarchimor 4260 + Mycorrhiza) and treatment four (Sarchimor 4260 + Trichoderma) show equality in percentage of shoot emergence during their evaluation period, resulting in treatment one (Sarchimor 1669 + Mycorrhiza) with the lowest number of shoot emergence during the 90-day period.

Table 1. *Percentage of grafted plants budding.*

Treatments	Prendimiento					
	30 days	%	60 days	%	90 days	%
T1 Sarchimor1669+Micorriza	15	94%	15	94%	12	75%
T2 Sarchimor 1669+Trichoderma	14	88%	14	88%	14	88%
T3 Sarchimor 4260+Mycorrhiza	16	100%	15	94%	14	88%
T4 Sarchimor 4260+Trichoderma	16	100%	15	94%	14	88%

Graft diameter

Table 2 shows the analysis of variance of the graft diameter, where statistical differences between treatments are determined by p-value<0.01; in the case of the interaction between treatment and time there is no difference, which determines a homogeneous behavior of all treatments during the time of the trial.

Table 2. *Analysis of variance of the variable graft diameter.*

Source of Variation	SC	gl	CM	F	p-value
Repetitions	0,11	3	0,04	4,12	0,0171
Treatments	1,1	3	0,37	41,32	<0,0001**
Weather	4,4	2	2,2	248,69	<0,0001**
Time*Treatments	0,07	6	0,01	1,28	0.3024 ns
Error	0,21	24	0,01		
Total	7,57	47			

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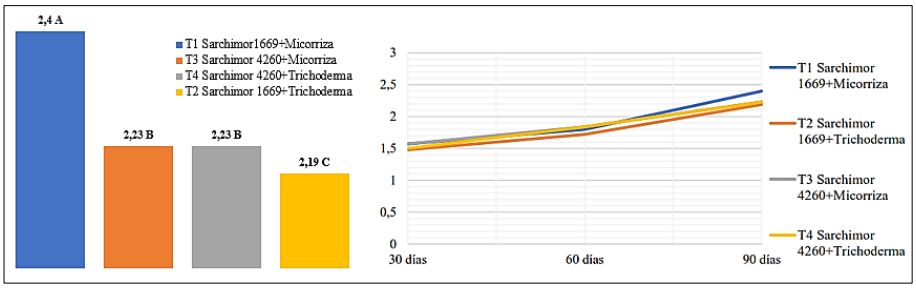
**= Highly significant at P<0.01, ns= not significant at P>0.05

The Tukey test at 5% determines that the treatment T1 Sarchimor 1669 + Mycorrhiza with 2.4 cm of diameter as the one with the best performance, and T2 Sarchimor 1669 + Trichoderma with the lowest average with 2.19 cm of diameter. With respect to the interaction of time, a homogeneous increase in the means was observed as time progressed, with an initial average of 1.48 cm diameter in the first 30 days and ending the evaluation period with 2.40 cm diameter average at 90 days (Figure 1).

Figure 1. *Graft diameter (cm) by treatments and time*



Graft length

In the analysis of variance of the graft length variable (Table 3), it was observed that there was a significant difference in the treatments, as well as a high significant difference with a p-value<0.01 for time. While for the interaction between treatments and time, there were no significant differences.

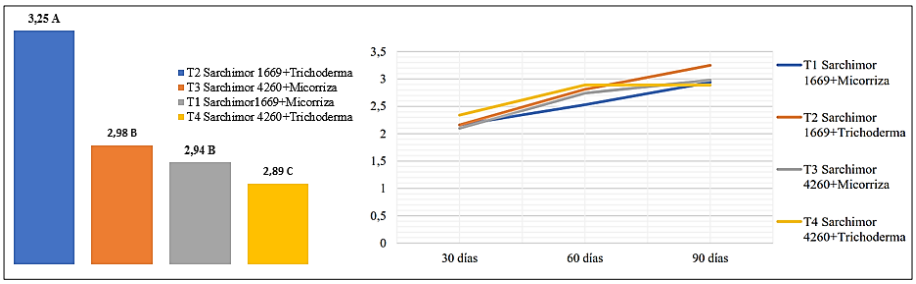
Table 3. *Analysis of variance of the variable graft length.*

F.V.	SC	gl	CM	F	p-value
Repetitions	1,90	3	0,63	16,17	<0,0001
Treatments	0,43	3	0,14	3,64	0,0304 *
Weather	5,54	2	2,77	70,7	<0,0001**
Time*Treatments	0,36	6	0,06	1,53	0.2115 ns
Error	0,94	24	0,04		
Total	10,08	47			

*= there is significance p<0.05, **= there is very high significance, ns= not significant P>0.05

The Tukey test at 5% in the length of the graft indicates that the treatment with statistically higher average was the T2 Sarchimor 1669 + Trichoderma with 3.25 cm, while the T4 Sarchimor 4260 + Trichoderma obtained 2.89 cm being the lowest average during its period of evaluation. With respect to time, a homogeneous increase is observed as time progresses, having an initial average of 2.10 cm in the first 30 days and ending the evaluation period with an average of 3.25 cm at 90 days (Figure 2).

Figure 1. *Graft diameter (cm) by treatment and time*



Number of sheets

Regarding the variable **number of leaves**, the analysis of variance (Table 4) showed that there are significant differences between treatments and time. In the case of the interaction between treatment and time, there is no difference with a p-value of 0.8209, being all equal during 90 days of evaluation significant differences (P<0.05 probability), T2 obtained an average of 23 nodes per plant with respect to T4, which had 20 nodes per plant.

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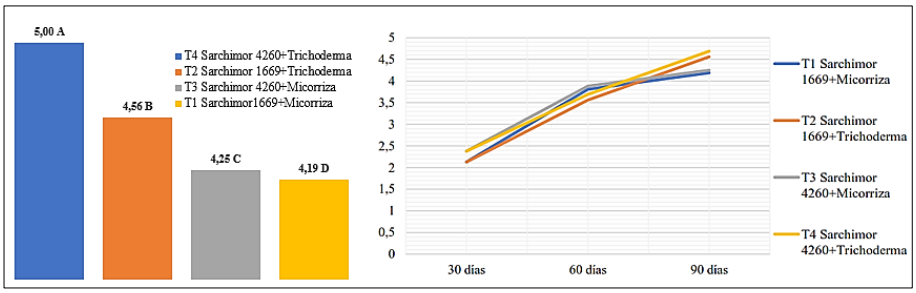
Table 4. *Analysis of variance of the variable number of leaves.*

F.V.	SC	gl	CM	F	p-value
Repetitions	3,23	3	1,08	3,51	0,0305
Treatments	3,17	3	1,06	3,44	0,0327*
Weather	39,43	2	19,71	64,25	<0,0001**
Time*Treatments	0,87	6	0,15	0,47	0.8209 ns
Error	7,36	24	0,31		
Total	59,58	47			

*= there is significance P<0.05, **= highly significant at P<0.01, ns= not significant P>0.05

The Tukey test at 5% of the variable number of leaves indicates that the treatment with the highest statistically dissimilar average was T4 Sarchimor 4260 + Trichoderma, while T1 Sarchimor 1669 + Mycorrhiza obtained the lowest average. With respect to the interaction of time, it shows that despite the increase in the number of days to complete the evaluation period, these remain homogeneous among themselves (Figure 3).

Figure 3. *Number of leaves per treatment and time.*



The results obtained in the present investigation determined that in the application of the queen grafting method 15 days after having carried out the same, 60% of the plants had taken root, likewise it was determined that treatment 3 (Sarchimor 4260 + Mycorrhiza) and treatment 4 (Sarchimor 4260 + Trichoderma) were the most efficient as opposed to the other treatments. On the other hand, Mallaguare (2021), reported a 60% of pruning using wedge grafting at nursery level. These values are similar to those of research. In contrast, Espinoza et al. (2017), using the queen grafting method, obtained a percentage of 95% bud break, reaching higher values.

During the 90-day evaluation period, there was a good development of the graft, characteristics that coincide with Piaguaje (2023), that the grafts show good plant vigor and little affectation in phytosanitary problems after 90 days of evaluation. According to Aguilar (2016), several factors must be taken into account that influence a graft to have positive results, among them are: temperature, humidity and compatibility.

Balón (2016), in his research shows that the application of Agriroot biostimulant in Arabica coffee grafting with robusta rootstock in doses of 200, 300 and 400 ml in an evaluation period of 15, 30 and 45 days, being the control T1 with the highest average of 1.86 cm and with the lowest average obtained the T3 Agriroot with doses of 300 ml with 1.80 cm in height.

According to Quijije (2021), it was necessary to make a trend line analysis with repetitions in the time of study that showed a similar progress during the time of the experiment. Pincay et at. (2022) expressed that the biostimulants affect the diameter of the stem, the treatment with *Trichoderma sp.* 4 ml L^{-1} was the most outstanding, followed by *Trichoderma sp.* 2 ml L^{-1} in three evaluations of 60, 90 and 120 days.

Franco & Vera (2018), in their research applying Induktor biostimulant in doses of 2.5 cc/liter in water, being the hybrid Sarchimor 4260 with the highest result in the variable number of leaves in average of 10 leaves at 90 days.

Conclusions

The morphometric characterization of the genotypes of arabica coffee studied indicate that Sarchimor 1669 + Micorriza presented better average in diameter of the graft, Sarchimor 1669 + Trichoderma obtained greater averages in length of the graft and as for number of leaves the Sarchimor 4260 + Trichoderma reached greater average of 5 leaves per plant. According to the analysis of the results obtained it is shown that the organic biostimulant *Trichoderma* acted in a positive way within the treatments . With respect to the time that was carried out, the investigation shows a homogeneous increase of the means as the time advances.

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