

# Effects Of The Use Of Cytokinins And Gibberellic Acid In The Cultivation Of Cocoa (Theobroma Cacao L.) Cultivar CCN-51 In Ecuador

Burgos Tanny <sup>1</sup>; Iler Víctor <sup>2</sup>; Villón Henry<sup>3</sup>;  
Pluas Cesar<sup>4</sup>

<sup>1</sup>ORCID: 0000-0003-4132-9905

Universidad Agraria del Ecuador

<sup>2</sup>ORCID: 0000-0003-0340-0811

Universidad Agraria del Ecuador

<sup>3</sup>ORCID: 0009-0007-6808-2697

Universidad Agraria del Ecuador

<sup>4</sup>Universidad Agraria del Ecuador

## ABSTRACT

The production of cocoa *Theobroma cacao* L. is essential for some developing countries, especially in South America, since it is an essential raw material in international trade operations, and in this context, improving production characteristics becomes relevant. The objective of this study was to evaluate the effects of the use of phytohormones in the pre-flowering stage of cocoa and their influence on production variables, including flowering and fertilization percentage, fruit length, kernel weight, number of fruits per plant and yield. A completely randomized design with five treatments and four replications was carried out, an analysis of variance and a comparison of means with Tukey's test  $\alpha=0.05$ . The results showed that the treatment with cytokinin's at a dose of 2.0cc/L (T3) obtained a CV of 3.54% and a p-value of 0.0016 in the applied model, reaching the highest yield 1287,5 kg/Ha being statistically equal to treatments T1, T2 and T4, but different from the control T5, on the other hand, the treatment that presented the best percentage of flowering and fertilization was the treatment with cytokinin's at a dose of 2.5cc/L (T1). It is concluded that the use of cytokinin's favors the percentage of flowering and fertilization in the cocoa crop, improving the yield compared to the control.

Keywords: Chocolate, flowering, phytohormones, production.

## INTRODUCTION

Cacao is a cash crop from the Amazon, whose trade is vital to the economy of many nations including Ecuador, as well as serving as

a raw ingredient in industrial operations. Cocoa is considered a luxury product in the almond business next to coffee, since it is not usually necessary for food; however, it is very important for the economies of developing nations in both South America and Africa; Because it has helped build very strong and growing economies, which is key for cocoa-producing countries. (Quintero R & Díaz Morales, 2004; Ramos et al., 2022)

Cocoa beans represent the essential raw material of chocolate, and are obtained from the *Theobroma cacao* L. tree, which is grown almost exclusively in tropical developing countries, where it represents a source of export earnings both at the level of families, communities and nations. Africa accounts for more than 75% of total global cocoa bean production and Côte d'Ivoire is the world's largest exporter of cocoa beans, with more than \$4 billion exported in 2020, followed by Ghana and Ecuador. (Ramos et al., 2022).

Phytohormones are regulators of plant growth and development, with very small and varied structures. Although plants synthesize them in small quantities, they are very active from the physiological point of view. According to their action, phytohormones can be divided into two categories, growth activators or inhibitors, auxins and cytokinins belonging to the first group (Sosnowski et al., 2023) . In addition, phytohormones are a key piece in the growth and development of plants, although they are also linked to the development of the defense system in plants. Although discovered in the twentieth century, with the passage of time and improvements in chemical techniques, more characteristics have been established that have allowed the development of agro-inputs, improving aspects of crop yield (Arévalo-Gardini et al., 2021; Coronado-Cáceres et al., 2021) .

Flowering is governed by hormonal signals that determine changes in each of the processes studied the genes involved in hormonal changes to maintain the flowering of Liu et al. (2023) *Hydrangea macrophylla* where he determined that they may be one of the complex hormonal regulatory networks involved in abscisic acid (ABA), auxin (IAA), nucleoside zeatin (ZR) and gibberellin (GA) who participate in the formation of flowers. (Cajamarca et al., 2017; Garay et al., 2014; Jean-Marie et al., 2022)

The objective of this study is to evaluate the effects of the use of plant hormones (cytokinins and gibberellic acid) on the reproductive status of cocoa trees (*T. cacao*) and their response in crop production and yield.

## METHODOLOGY

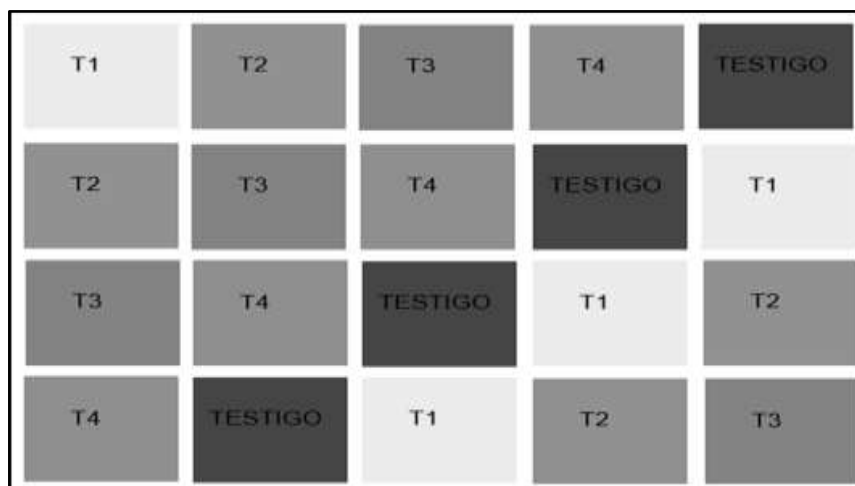
The present study was carried out in the sector of San Antonio located in the province of Los Ríos, Montalvo canton whose UTM (Universal Transversal Mercator) coordinates are X: 690339 Y: 9807305.

To determine the influence of the use of cytokinins and gibberellic acid in cocoa cultivation, the following variables were evaluated: Flowering percentage (%), fertilization percentage (%), fruit length (cm), number of fruits per plant (n), number of grains per ear (n), Weight of 100 almonds (gr), Yield (kg/ha). The treatments with the different doses of plant hormones were distributed as shown in Table 1.

**Table 1:** Distribution of treatments

Treatment	Active ingredient	Dose	Frequency
T1	Cytokinin	2.5 cc/L	21 DAF (Days before flowering)
T2	Gibberellic acid	50.0 mg/L	21 DAF (Days before flowering)
T3	Cytokinin	2.0 cc/L	21 DAF (Days before flowering)
T4	Gibberellic acid	40.0 mg/L	21 DAF (Days before flowering)
T5	Witness	Not applicable	Not applicable

Plots were systematically established for each of the treatments as can be seen in Figure 1. Each plot has an area of 384m<sup>2</sup>, being a total of 25 plots for the 5 treatments with their 4 respective repetitions, within each plot there is a total of 32 cocoa trees.



**Figure 1:** Sketch of the experimental trial

In each plot, 5 trees were randomly selected, with an approximate age of 4 years; The climatic conditions during the test were as follows: Average temperature of 26°C, average relative humidity of 75%, annual precipitation of 1750 mm.

### Test procedure

To evaluate each of the variables, the number of flowers was counted 21 days after the application of plant hormones based on the defined treatments; the percentage of flowering was evaluated based on the number of fertilized and unfertilized flowers; the length of fully developed fruits was measured; the number of fruits per plant was counted; The number of grains per ear and the fresh weight of 100 almonds were recorded in order to estimate the crop yield.

### Statistical analysis

The statistical analysis consisted of a single-factor ANDEVA for each of the variables under study, to perform the comparison of means a Tukey test with 5% significance was applied . The software used was InfoStat (2020).

### RESULTS

Based on the test performed with plant hormones in the reproductive state of the cocoa crop (*T. cacao*) significant results were obtained. The percentage of flowering in cocoa trees presented a coefficient of variation (CV) of 1.93% with a p-value of 0.0017 in the applied model, evidencing significant differences between the treatments, in terms of comparison of means, as can be seen in Table 2 the use of gibberellins at a concentration of 50.0 mg / L T2 is statistically equal to the control, while the other corresponding treatments presented significant differences presenting a greater number of flowers compared to the control.

**Table 2:** Comparison of means (Tukey  $\alpha = 0.05$ ) in the percentage of flowering.

Treatment	Half	
T1 Cytokinins (2.5 cc/L)	239,40	to
T3 Cytokinins (2.0 cc/L)	236,80	to
T4 Gibberellins (40.0 mg/L)	236,25	to
T2 Gibberellins (50.0 mg/L)	226,10	B
T5 Witness	222,90	B

Note: Stockings with a letter in common are not significantly different

On the other hand the percentage of fertilization of the flowers obtained a CV of 3.53% with a p-value of 0.0081 in the applied model, concluding that there is a statistical difference between the treatments evaluated, in the comparison of means that can be observed in table 3 it is established that the treatment with the largest number of fertilized flowers is T1 with the use of cytokinins in doses of 2.5 cc / L, while the T3 and T4 treatments present similarity to the T1 treatment, however, all treatments were different from the T5 control.

**Table 3:** Comparison of means (Tukey  $\alpha = 0.05$ ) in the percentage of fertilization.

Treatment	Half			
T1 Cytokinins (2.5 cc/L)	11,25	to		
T3 Cytokinins (2.0 cc/L)	11,00	to	b	
T4 Gibberellins (40.0 mg/L)	10,75	to	b	c
T2 Gibberellins (50.0 mg/L)	10,25		b	c
T5 Witness	10,00			c

Note: Stockings with a letter in common are not significantly different

The amount of fruits per plant presented a CV of 2.49% with a p-value of 0.0001 for the applied model, showing significant differences between treatments; in the comparison of means that we can observe according to Table 4, the treatment with the highest number of fruits was T1 with the use of cytokinins at a dose of 2.5cc / L, in addition the control presented the lowest average for the number of fruits. A key factor in establishing differences in the influence of plant hormone uses in cocoa cultivation.

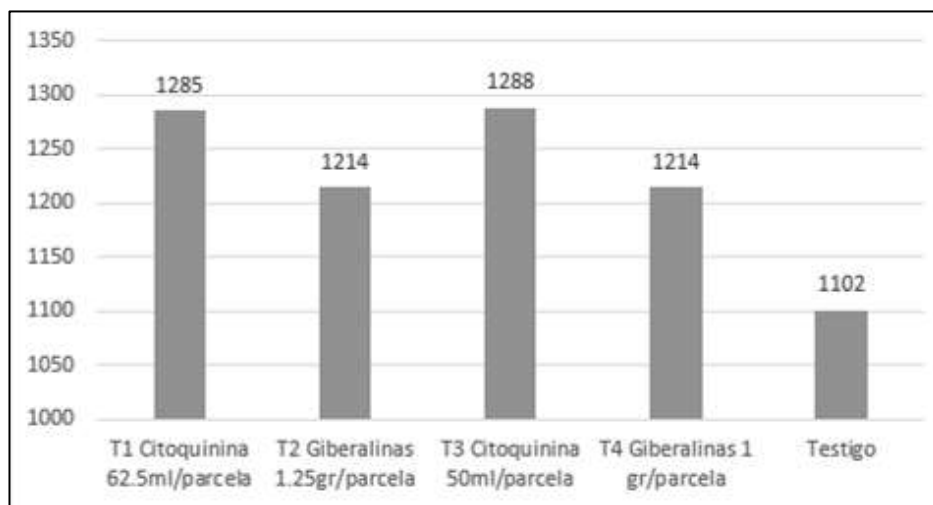
**Table 4:** Comparison of means (Tukey  $\alpha = 0.05$ ) in the number of fruits per plant.

Treatment	Half			
T1 Cytokinins (2.5 cc/L)	26,00	to		
T3 Cytokinins (2.0 cc/L)	25,50	to	B	
T4 Gibberellins (40.0 mg/L)	25,00	to	B	
T2 Gibberellins (50.0 mg/L)	24,25		B	
T5 Witness	22,25			c

Note: Stockings with a letter in common are not significantly different

The yield, one of the most important variables, obtained a CV of 3.54% and a p-value of 0.0016 in the applied model, showing significant differences between the treatments, of which, according to the comparison of means the treatment with the highest performance in the trial was the T3 with an average of 1287.50 kg / ha, although being significantly the same as the other treatments except for the control as we can see in figure 2.

It should be noted that, for the variables of number of grains per ear and weight of 100 fresh almonds, no statistically significant differences were evident.



**Graph 2:** Yield in kg/Ha of the cocoa crop evaluated.

## DISCUSSION

The use of plant hormones in agriculture aims to meet the constant demand for food that grows exponentially, its importance is based on contributing to global food security; That is why alternatives to improve crop production and yields become increasingly necessary. (Borjas et al., 2020).

(2019)The results show that the implementation of plant hormones, in this case cytokinins and gibberellins promote the percentage of fertilization in cocoa flowers, which leads to an increase in yield since greater fruits come to term at the time of harvest. This agrees with Urrutia who in his experimental work determined that each hormone fulfills a specific function for the growth and production of crops; The use of gibberellins provides the ability to develop tissues and cytokinins give plants the ability to improve fruit formation and stimulate fruit growth and

development through cell division. (Mok & Mok, 2001; Thimann, 1977).

The amount of fruits per plant was higher in the treatments with the use of plant hormones compared to the control; the results shown by the study according to the amount of fruits per plant where it specifies that the Treatment 1 Cytokinin 62.5 ml gives us an average of 26 fruits per plant, this being with more significance with respect to the control that gave us a result of 22.25 fruits per plant and agrees with what was exposed by, where it indicates that cytokinins slow down the separation and senescence of flowers, fruits and leaves, promotes the formation of flower buds and improves fruit formation. Cytokinins, in turn, are Liu et al. (2023)

They form at the tips of the roots, in the fruits and immature seeds. These hormones are responsible for stimulating the growth of lateral shoots, they also stimulate cytokinesis and, consequently, cell division (Sosnowski et al., 2023) .

The use of cytokinins has been shown to have positive effects on improving plant tolerance to heat stress, salinity and drought. Increases the expression of antioxidant enzymes, and genes involved with the plant defense system, balancing physiological processes that can result in benefits for crop production and yield. (Mei et al., 2023).

### **CONCLUSIONS**

It is determined that the effects on floral activity and fertilization were positive for the T1 treatment that shows the best results in these two variables; All treatments with the use of plant hormones were statistically significant compared to the control. The treatment that obtained the best results in performance was T3 which consists of the use of cytokinins at a dose of 2.0cc/L reaching 1287.5 kg/Ha, above that achieved by the control treatment of 1102.0 kg/Ha.

This paper reflects the notable differences in the implementation of hormonal regulators to influence the reproductive behavior of the cocoa crop, impacting with the results on yield and therefore productivity. They become promising alternatives, since, when synthesized in small proportions within the plant, the doses used in agro-inputs are small.

## BIBLIOGRAPHIC REFERENCES

- Arévalo-Gardini, E., Farfán, A., Barraza, F., Arévalo-Hernández, C. O., Zúñiga-Cernades, L. B., Alegre, J., & Baligar, V. C. (2021). Growth, physiological, nutrient-uptake-efficiency and shade-tolerance responses of cacao genotypes under different shades. *Agronomy*, 11(8), 1536.  
<https://doi.org/10.3390/AGRONOMY11081536/S1>
- Borjas, R., Julca, A., & Alvarado, L. (2020). Phytohormones are a key part of the development of agriculture. *Journal of the Andean Forest Biosphere*, 8(2), 150–164.  
[http://www.scielo.org.bo/pdf/jsab/v8n2/v8n2\\_a07.pdf](http://www.scielo.org.bo/pdf/jsab/v8n2/v8n2_a07.pdf)
- Cajamarca, E., Quevedo, J., & García, R. (2017). Efficiency of hormones in the rooting of cocoa twigs (*Theobroma cacao* L.) national x trinitarian type. *Scientific Journal Agroecosystems*, 5(1), 6–15.  
<https://www.researchgate.net/publication/323200003>
- Coronado-Cáceres, L. J., Hernández-Ledesma, B., Mojica, L., Quevedo-Corona, L., Rabadán-Chávez, G., Castillo-Herrera, G. A., & Cervantes, E. L. (2021). Cocoa (*Theobroma cacao* L.) Seed-Derived Peptides Reduce Blood Pressure by Interacting with the Catalytic Site of the Angiotensin-Converting Enzyme. *Foods* 2021, Vol. 10, Page 2340, 10(10), 2340.  
<https://doi.org/10.3390/FOODS10102340>
- Garay, A., De La Paz Sánchez, M., García, B., Álvarez, E., & Gutiérrez, C. (2014). Auxin homeostasis and its importance. *REB*, 33(1), 13–22. <https://www.scielo.org.mx/pdf/reb/v33n1/v33n1a3.pdf>
- Jean-Marie, E., Jiang, W., Bereau, D., & Robinson, J. C. (2022). *Theobroma cacao* and *Theobroma grandiflorum*: Botany, Composition and Pharmacological Activities of Pods and Seeds. *Foods* 2022, Vol. 11, Page 3966, 11(24), 3966.  
<https://doi.org/10.3390/FOODS11243966>
- Liu, Y., Lyu, T., & Lyu, Y. (2023). Study on the Flower Induction Mechanism of *Hydrangea macrophylla*. *International Journal of Molecular Sciences* 2023, Vol. 24, Page 7691, 24(9), 7691.  
<https://doi.org/10.3390/IJMS24097691>
- Mei, W., Chen, W., Wang, Y., Liu, Z., Dong, Y., Zhang, G., Deng, H., Liu, X., Lu, X., Wang, F., Chen, G., Tang, W., & Xiao, Y. (2023). Exogenous Kinetin Modulates ROS Homeostasis to Affect Heat Tolerance in Rice Seedlings. *International Journal of Molecular Sciences*, 24(7), 6252.  
<https://doi.org/10.3390/IJMS24076252/S1>
- Mok, D. W., & Mok, M. C. (2001). Cytokinin Metabolism and Action. In *Annu. Rev. Plant Physiol. Plant Mol. Biol* (Vol. 52).  
[www.annualreviews.org](http://www.annualreviews.org)



- Quintero R, M. L., & Díaz Morales, K. M. (2004). The world cocoa market. *Agrifood*, 9(18), 47–59.  
[http://ve.scielo.org/scielo.php?script=sci\\_arttext&pid=S1316-03542004000100004&lng=en&nrm=iso&tlng=en](http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S1316-03542004000100004&lng=en&nrm=iso&tlng=en)
- Ramos, C., Ruales, J., Rivera-Parra, J. L., Sakakibara, M., & Díaz, X. (2022). Sustainability of Cocoa (*Theobroma cacao*) Cultivation in the Mining District of Ponce Enríquez: A Trace Metal Approach. *International Journal of Environmental Research and Public Health* 2022, Vol. 19, Page 14369, 19(21), 14369.  
<https://doi.org/10.3390/IJERPH192114369>
- Sosnowski, J., Truba, M., & Vasileva, V. (2023). The Impact of Auxin and Cytokinin on the Growth and Development of Selected Crops. *Agriculture* 2023, Vol. 13, Page 724, 13(3), 724.  
<https://doi.org/10.3390/AGRICULTURE13030724>
- Thimann, K. (1977). *Hormone action in the whole life of plants*. University of Massachusetts Press. <https://doi.org/10.3/JQUERY-UI.JS>
- Urrutia. (2019). Application of trihormonal biostimulants in the cultivation of corn (*Zea mays* L.) variety chingasino for corn yield. [Thesis]. National University of Central Peru.