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Calibration of cocoa orchard (*Theobroma cacao* L.) with different reproductive methods and their effect on climate change

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Introduction

The cocoa plant is crucial for the economy and livelihoods of millions of farmers in tropical regions, but its production is threatened by climate change. Traditional propagation practices, such as seed planting, fail to ensure the necessary uniformity and resilience against these changes, highlighting the need to explore alternative methods like grafting and cloning. These methods could enhance cocoa's adaptation to adverse climate conditions and reduce its carbon footprint. This study aims to compare different reproductive methods in terms of production, disease resistance, and climate adaptation (Vera et al., 2023).

The top eight cocoa-producing countries, ranked by their annual production, are Ivory Coast (38%), Ghana (19%), Indonesia (13%), Nigeria (5%), Brazil (5%), Cameroon (5%), Ecuador

(4%), and Malaysia (1%). Together, these countries account for 90% of the world's cocoa production. In Ecuador, most of the cocoa cultivation is concentrated in the provinces of Los Ríos, Guayas, Manabí, and Sucumbíos. The primary export markets for Ecuadorian cocoa are Europe (55%), the United States (35%), Asia (9%), with the remainder distributed within Latin America (Vásquez et al., 2022).

Climate change, evidenced by a 1 °C increase in global temperature since the pre-industrial era, affects cocoa production, with projections of a 3 °C to 5 °C increase by 2100 (BBC News World, 2018). ICTA (International Center for Tropical Agriculture) has developed a climate change impact gradient for cocoa, predicting that a temperature increases of 2.1 °C will result in decreased production due to moisture loss. This could lead to a cocoa shortage and an increase in prices for the product and its derivatives, such as chocolate. Therefore, studying orchard calibration in cocoa and its relationship with climate change is crucial for understanding and mitigating these effects. The main objective of the research study is to determine the calibration of cocoa orchards (*Theobroma cacao* L.) Nacional, Forastero, and Trinitario as a diagnostic tool for climate change.

Materials and Methods

Location

This research was conducted at the Experimental Farm "La María" located at the Technical State University of Quevedo. Bromatological evaluation took place at the Bromatology Laboratory of the same institution (Campus La María, Los Ríos Province, Ecuador). Organoleptic evaluation was conducted at the Comprehensive Cocoa Quality Laboratory of the National Institute of Agricultural Research - Pichilingue (Quevedo, Los Ríos Province, Ecuador) (Erazo et al., 2023).

Methods

A Completely Randomized Design (CRD) was employed, comprising 12 treatments classified into 3 distinct types, each replicated 4 times as indicated in Table 1. Experimental units consisted of 20 plots, and repetitions were conducted monthly during harvests to assess differences among treatment means. Statistical analysis included the Tukey test for multiple comparisons with a significance level of $P<0.05$ (Vera, 2018).

The Tukey test, or Tukey's honestly significant difference (HSD) test, is a statistical procedure used to make pairwise comparisons between means of different groups following the detection of significant differences in an analysis of variance (ANOVA). This test calculates confidence intervals for the differences between all possible combinations of treatment means (Vera et al., 2022).

Table 1
Identification and coding of the 12 treatments to be evaluated

Block	Treatment	Number of replications	Total number of units
2	T0 (Forastero)	4	24
2	T1 (Trinitario)	4	24
2	T2 (Nacional)	4	24
Total		12	72

Number of healthy pods (NHP) and number of diseased pods (NDP)

The total number of healthy and physiologically mature pods per tree will be counted according to the harvest frequency. Similarly, for the healthy pods, the diseased pods were counted and separated into different containers (Vera & Salazar, 2021).

Total pods (TP)

In this variable, all pods (healthy and diseased) were counted during the harvest period. For this, a harvest record was used, which involved counting the number of pods from selected plants in each experimental unit, and then averaging the data (Quezada et al., 2017).

Performance

Equation 1. Perfomance cocoa

$$P = \left(\frac{\mathrm{Nm}}{\mathrm{IM}}\right) \times \mathrm{Np}$$

It was determined using the following formula:

Where:

P= Performance
Nm= Number of cobs per plant
IM = Ear Index.
Np = Number of plants per hectare.

Cob morphology evaluations

This is the number of mature and healthy pods of each genotype needed to obtain one kilogram of dry cocoa. The following formula was used for its calculation:

The Cob index was determined by assessing 20 physiologically mature cobs, free from any disease symptoms, in each sample (randomly selected). Following almond extraction, the almonds were subjected to fermentation and drying until they reached 7% humidity (Vásquez et al., 2023). The Cob index was calculated using the specified formula

Equation 2. Cacao cob index (CCI)

$$\mathrm{IM} = \frac{Number\ of\ 20\ cobs \times 100}{Weight\ (g) of\ dried\ almonds}$$

Cob weight was recorded with a digital scale. Length and diameter were recorded with a ruler (diameter from furrow to groove from the equatorial diameter). Cob ridge and spine thickness were recorded with a caliper. The Seed index were performed taken at random 300 fermented and dried almonds; all almonds were weighed, and averages were calculated. The following formula was applied:

Equation 3. Seed index

$$\mathrm{SI} = \frac{\text{Weight in grams of 300 fermented and dried seeds}}{300}$$

Cobs Length

Ten cobs randomly selected from each treatment are measured using a caliper to calculate the average (Lagunes et al., 2018).

Cobs Width

After harvesting, 20 cobs were randomly selected from each treatment and then weighed using a precision scale, and the weight was recorded (Chacón et al., 2007).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was applied to obtain scatter plots (biplots) of the quantitative variables grouped according to their optimal components of the productive and physical profiles, using the following formula:

Equation 4. Component Analysis

$$rij = \frac{cov(F1,Fj)}{\sqrt{var(Fi)var(Fj)}}$$

Where:

Cov. X, Y = Covariance of Fi and Fj
S^2Fi = Variance of X
S^2Fj = Variance of Y

Principal Component Analysis (PCA) and biplots are used in dimension reduction, allowing for the visualization of all generated data in a lower-dimensional space. Artificial axes, known as Principal Components, are constructed to analyze and synthesize the variability or dispersion with their original characteristics. Biplots graphically express this information to identify associations. The new principal components or factors are linear combinations of the original variables and are independent of each other. The results are presented in graphical form (biplot) (Vera et al., 2014).

Results

Climatic variables

This comparison of the means of the climatic data for the three years indicates the following: The average air temperature in degrees Celsius for the year 2017 is 24.9 °C, higher than for the year 2018 at 24.55 °C, and lower than for the year 2019 at 24.97 °C, making it the highest average temperature of the three years. For the maximum temperature, 2017 recorded 30.06 °C, the highest of the three years, with 2018 being the lowest at 29.53 °C, and 2019 at 29.85 °C. Similarly, the minimum temperature for 2017 is 22.18 °C, followed by 21.71 °C for 2018, the lowest minimum of the three years, and 22.07 °C for 2019. The temperature range for 2017 is 7.89

°C, the highest value, followed by 7.81 °C for 2018, and 7.19 °C for 2019, the lowest value (Table 2).

The average relative humidity for the year 2017 was 85.58%, for 2018 it was 84.33%, the lowest average, and for 2019 it was 85.83%, indicating the year with the highest average value. Consequently, the maximum humidity for the years 2017 and 2019 was 96.83%, while for 2018 it was 96.33%. Finally, the minimum humidity for 2017 has a value of 62.25%, the lowest, followed by 62.75% for 2018, and 64.50% for 2019, indicating the highest minimum humidity (Table 2).

Table 2
Annual climatic conditions during the years (2017-2019) of T. cacao progeny evaluation in the Quevedo area of Ecuador

Weather Conditions		Experimental Farm ‘La Represa			Average
		2017	2018	2019	
Air Temperature (°C)	Mean	24.90	24.55	24.97	24.81
	Maximum	30.06	29.53	29.85	29.81
	Minimum	22.18	21.71	22.07	21.98
	Oscillation	7.89	7.81	7.19	7.63
Relative Humidity	Mean	85.58	84.33	85.83	85.25
	Maximum	96.83	96.33	96.83	96.67
	Minimum	62.25	62.75	64.50	63.17
	Annual Sunshine Duration	73.38	67.10	66.96	69.15
	Evaporation (mm)	86.54	92.41	81.27	86.74
	Precipitation (mm)	272.56	144.22	249.98	222.25

Note: Three-year averages of the weather conditions are displayed

Production metrics variables

In the obtained results, regarding the number of healthy cobs, number of diseased cobs, and total cobs presented in Table 3, it was observed that there was no significant difference ($p \leq 0.05$) between the treatments for the aforementioned variables. Regarding the yield, due to significant difference ($p<0.05$), it was demonstrated that T2 achieved the highest yield with 2245.42 kg/ha/year, while T3 showed a lower yield of 1681.60 kg/ha/year

Table 3
Statistical averages of cocoa (T. cacao) production parameters

Treatments	NHP	NDP	TP	Yield per hectare per year calibrated	Production per hectare per year
T0	12.02[a]	2.65[a]	14.67[a]	1864.25[b]	1219.52[b]
T1	14.06[a]	2.62[a]	16.43[a]	2245.42[a]	1284.14[a]
T2	11.69[a]	2.36[a]	14.31[a]	1681.60[c]	1015.52[c]
Average	12.59	2.54	15.14	1930.42	1173.06
C.V.	0.10	0.06	0.07	0.15	0.12

Note: The calibrated yield is shown compared to the yield per hectare per year.

Fruit parameters

In the cob index, no significant difference ($p \geq 0.05$) was found among the studied treatments; however, the highest value was observed in T3 (23.17), while T2 had the lowest value (20.87). Regarding the seed index variable, there was no statistical significance ($p \geq 0.05$) between treatments T0 and T2 with a value of 1.17, which, unlike T2, indicates statistical significance with a higher value (1.22). In relation to cob weight, it was observed that there was no statistical significance among the treatments.

Table 4
Parameters of Nacional, Forastero, and Trinitario cocoa fruit (PI; Pod Index, SI; Seed Index, PW; Pod Weight)

Treatment	PI	SI	PW
T0	21.49[a]	1.17[a]	701.38[a]
T1	20.87[a]	1.22[b]	749.50[a]
T2	23.17[a]	1.17[a]	691.38[a]
Average	21.18	1.39	714.09
C.V.	2.1%	24%	4.4%

Fruit

Calibration

Regarding the length of the cocoa pod, in the first and fourth months of growth, there was no statistical significance ($p \geq 0.05$) among the studied treatments. However, in the second month, it was demonstrated that T2 with 10.39 was statistically different ($p \geq 0.05$) from T0, which had the smallest size. Additionally, in the fifth month (harvest month), there was statistical significance indicating the highest value in T0 with 24.15 and the lowest value in T2 with 22.30.

In relation to the width of the cocoa pods, in the first, fourth, and fifth months, the treatments did not present significant differences (p≥0.05). However, in the second month, the treatments showed significant differences (p≥0.05), with the highest value in T2 (4.88) and the lowest value in T1 (2.81), with an average of 3.58 and a coefficient of variation of 31.7%. As for the third month, it was determined that T0 and T2 with 7.9 were statistically different (p≥0.05) from T2 with 6.28, which obtained a lower value.

Principal Component Analysis (PCA)

Through PCA, a comparison was made between physical, productive, and climatic parameters. It was found that physical quality parameters such as IS (Seed Index), IM (Pod Index), and AM (Pod Weight) have a greater influence, while temperature and humidity have a lesser influence on Nacional variety. In contrast, Forastero variety shows a higher contribution from temperature, humidity, and the number of pods. Regarding the Trinitario variety, pod weight is related to calibrated yield, closely followed by actual yield and pod length. Considering the sum of these two components, total of 100% of the existing total variability was obtained, allowing for a more comprehensive relationship.

The climate exhibits a moderate range for cocoa cultivation; however, certain months of the year experience high peaks of temperature and maximum precipitation, which have influenced the presence of pests such as monilia. The terrain in all analyzed systems shows flat surfaces, with slopes less than 2%, indicating a low risk of soil erosion (Albiño, 2020). Despite Criollo cocoa being a variety with a unique genotype known for its fine flavor chocolate, more vigorous hybrids with higher agronomic yield and lower susceptibility to diseases, such as Trinitario, have been introduced (Mejía et al., 2018).

Table 5
Fruit calibration of Nacional, Forastero, and Trinitario in each of their stages

Treatments	COBS									
	1° Month		2° Month		3° Month		4° Month		5° Month	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
T0	6.30ᵃ	2.05ᵃ	8.55ᵇ	3.04ᵃᵇ	15.28ᵃ	7.90ᵃ	18.58ᵃ	8.29ᵃ	24.15ᵃ	9.70ᵃ
T1	6.48ᵃ	1.95ᵃ	9.39ᵃᵇ	2.81ᵇ	14.99ᵃ	7.88ᵃ	19.59ᵃ	8.45ᵃ	23.59ᵃᵇ	10.33ᵃ
T2	6.16ᵃ	1.89ᵃ	10.39ᵃ	4.88ᵃ	14.52ᵃ	6.28ᵇ	19.55ᵃ	8.54ᵃ	22.30ᵇ	9.13ᵃ
Average	6.313	1.96	9.44	3.58	14.93	7.35	19.24	8.43	23.35	9.72ᵃ
C.V. (%)	2.5%	4.1%	9.8%	31.7%	2.6%	12.6%	3.0%	1.5%	4.1%	6.2%

Note: This table shows the calibration or measurement of pods during the five months of the stud

Discussion

Climatic variables

The climatic conditions, as determined in the study, are within the parameters established by Zamora, (2018), who, in their research, emphasize the importance of considering climatic conditions in order to prevent and reduce soil erosion, both in the agricultural sector and in deforestation

Production metrics variables

The data reported on the number of healthy cobs is similar to what was determined by Erazo et al. (2023), who indicated a small production (30-194 healthy cobs) in clones T23, INIAP 484, and T13 during the months of March-April, with a peak in September. Clones T11, T8, and T1 demonstrated the highest production of healthy cobs, while the least productive clones were T23, INIAP 484, and T13. Microorganisms used as control methods have been found to be highly efficient in reducing the two most devastating diseases in cocoa crops (moniliasis and witches' broom), as evidenced by the literature. Villamil et al. (2015) indicate that the most commonly used biocontrol agents are fungi (*Trichoderma* sp.) and bacteria (*Bacillus* sp.), which undergo metabolic processes and can be used for biological control.

In the study conducted by Tezara et al. (2020), it was reported that the most vulnerable clones with the highest rate of diseased cobs were T23, INIAP 484, T24, and T13 (50-60%). Meanwhile, the least susceptible clones were T11, PMA 12, T8, and T1 (5-27%). In contrast, clones CCN51 and T1 had the highest number of diseased cobs, as found by Cortez et al. (2017), while those showing the lowest number of diseased cobs were clones T8, PMA 12, and T11 respectively. According to Vera & Goya, (2015),there are manyFactors that affect the final number of fruits, including "cherelle wilt" or premature death, which can destroy fruits in their early stage and reduce them by 20 to 90%.

According to the National Institute of Forestry Research (2019), the Chocotab hybrid has been statistically superior to its parents, with an average of 45 fruits/plant/year over five years of evaluation. Its fruit index is 22, similar to UF 273, but higher than PA 169 with 26 fruits. (Vera et al., 2023) affirm that the number of present cobs is not a good indicator of yield because many cobs from some trees produce more cocoa seeds than others.

Fruit parameters

The cob index is a significant trait in the industry and in the selection of material for genetic improvement, with a preference for materials with an index lower than 20 cobs (Vera et al., 2022).

According to Vera et al. (2019), the physical quality of almonds in twenty-one interclonal cocoa crosses in Ecuador ranged from 1.41 to 0.97, with an average of 1.25. Intriago et al. (2023) indicate that there is variability among genotypes related to this index; Trinitario-type cocoas have a lower seed index than Forastero type.

Seed weight is related to findings by (Garcia et al., (2019), where the average cob weight and percentage of shell were 732.8 g and 81.8%, respectively. It is worth noting that the cocoa shell percentage can vary from 52% to 76%. According to the National Institute of Forestry Research, average weights range from 261g to 454g. On the other hand, Álvarez et al. (2020), mention a relationship between the degree of cob ripeness and grain weight; the riper the cob, the heavier the grains.

Fruit calibration

According to Quevedo Guerrero, (2018), in their studies conducted on fifth-month cocoa pods in the phenotypic characterization, they determined an average fruit length of 20.8 ± 0.88, indicating similar results to those obtained in this study. Additionally, they reported a fruit width of 9.10.

Conclusions

The productive parameters based on NMS, NME, and MT of each variety did not present significant differences according to the Tukey test ($p \geq 0.05$), obtaining average values of 12.59, 2.54, and 15.14, respectively. With these data, a calibrated yield was provided, which showed statistical significance among its treatments, with the highest value in T2 (2245.42 kg/ha/year) and the lowest value in T2 (1681.60 kg/ha/year). These results were obtained when the environment had a maximum temperature of 29 °C, a minimum of 22.07 °C, and an average of 24.97 °C, with an average humidity of 85.83%.

The evaluation based on the physio-phenological characteristics of cocoa revealed an average total pod count of 15.14, including healthy and diseased pods across treatments. It is worth mentioning that there was moderate to low production during the study months. Regarding calibration, it was determined that in the second month, pods showed statistical significance among treatments in length and width, with the highest value in T2 (10.39 - 4.88), the lowest value for length in T0 (8.55), and for width in T1 (2.81). For the third and fourth months, there was no significant difference, but in the fifth month, pods indicated the following: for length, the highest value was in T1 (24.15) and the lowest in T2 (22.03), and for width, the highest value was in T1 (10.30) and the lowest in T2 (9.13).

To validate this calibration process, a PCA was conducted to compare the physical, productive, and climatic parameters. It was found that calibration tends to be more accepted by the Trinitario variety, while the variety most related to climate is Forastero, as obtained in this research. Each treatment was rigorously selected to identify the evaluated varieties, determining their accuracy and reliability in the entire process.

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