AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Reproductive and morphological phenology of eight Mexican cacao clones (*Theobroma cacao* L.)

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ABSTRACT

Objective: To describe the dynamics of floral biology and fruit development until physiological maturity, by performing a qualitative and quantitative characterization in eight Mexican cacao clones (*Theobroma cacao* L.), called: Caehui, Chak, Canek, Chibolon, Supremo, Tabscoob, K'in and Olmeca.

Design/methodology/approach: In the phenological study, five trees were taken into account and five flower buds per tree were randomly identified (n=225). Twenty-one (21) qualitative and quantitative morphological descriptors were evaluated in fruits and grains. Descriptive statistics were established and a principal component analysis was applied to the quantitative descriptors.

Results: The Chak clone presented the highest value for length and diameter of flowers buds in reproductive phenology, with 7.4 mm and 4.2 mm; the K'in clone stood out in fruits, with 252 mm length; and Supremo in fruit diameter, with 102 mm. Significant differences were found in the morphological variables evaluated, in addition to significant positive correlation ($p<0.05$ and $p<0.001$) between most of the variables. The first two main components described 62.5% of the total variation.

Findings/conclusions: The differences in measurements (length and diameter) of the flower buds are attributable to the genetic constitution; however, knowing the opening times allows us to have an exact reference in production levels. The parameters that contributed most to the variability observed were weight and diameter of the fruit, length and width of the grain, thickness of the exocarp, and weight of grains per pod.

Keywords: cacao beans, clones, growth dynamics, descriptors.

Citation: Rivera-Torrez, D. L., Lagunes-Gálvez, L. M., Azpeitia-Morales, A, & García- Alamilla, P. (2022). Reproductive and morphological phenology of eight Mexican cacao clones (*Theobroma cacao* L.). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i8.2204

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: January 19, 2022.
Accepted: June 17, 2022.
Published on-line: September 15, 2022.

Agro Productividad, 15(8). August. 2022. pp: 157-167.

INTRODUCTION

Genetic diversity in cacao contributes to the sustainability of the global economy of cacao, with particular interests that range from production, resistance, nutraceutical aims, product elaboration, to the selection of cacao that fulfills quality requirements (Aikpokpodion, 2010). Morphology and geographical origin have been defining parameters in the description of genetic groups of cacao, where phenotypical differences

Image by David Greenwood-Haigh at Pixabay due to genetic variability are observed, as well as qualitative and quantitative differences in the indicators of cacao fruits. Studies report morphological variations in fruits of Criollo, hybrid cacaos and international reference material, showing the heterogeneity between plantations. The morphological characteristics of the pods, seeds and flowers have also been used to evaluate the relationships between cacao genotypes (Ramírez *et al.*, 2018; Ph *et al.*, 1999). A study conducted by López-Hernández *et al.* (2018) reported variability in length and diameter in buds and fruits of clones, and Aikpokpodion (2010) reported 43.6% of the total variation observed in qualitative and quantitative ranges of fruits. On the other hand, Bekele *et al.* (2006) obtained significant variations in morphology in studies about cacao accessions. Part of the descriptions of differentiation and characterization stems from the reproductive phenology in cacao until maturity, the varieties tend to have different characteristics in their flowering, appearance of flower buds and fructification (Santos *et al.*, 2012; Tabla & Vargas, 2004). These descriptions provide growth patterns in an objective and unambiguous manner, as indicated by Melgarejo (2015), mentioning that with bud measurements the trends in the shapes of fruits can be known according to the length and diameter ratio. Therefore, the use of tools for qualitative and quantitative descriptors allows obtaining elements that highlight the potential of new clones in relation to cacao with quality, productivity and yield. Anita-Sari, I., & Susilo (2015) highlighted the importance of the growth cycle with the yield and formation of fruits, and in their studies, Marcano *et al.* (2009) determined the mixture mapping of the entire genome of cacao groups for yield factors and morphological traits, defining the characterization of the first link in the amplitude of the variation and the trend towards phenotypical characteristics. Therefore, the research objective was to describe the floral biology and fruit development until physiological maturity, in addition to their qualitative and quantitative characterization in eight new clones generated in the germplasm garden of the INIFAP Experimental Field in Huimanguillo, Tabasco, Mexico.

MATERIALS AND METHODS

Variables for flower buds and fruits: Eight clones were evaluated for this study: Caehui (UF 613×IMC 67), Canek (RIM 75×SPA 9), Chak (RIM 76A×EET 48), Chibolón (UF 613×Pound 7), K'in (CC 226×IMC 67), Olmeca (RIM 76A×EET 400), Tabscoob (RIM 76A×EET 48×PA 169) and Supremo (UF 613×IMC 67), in addition to a control called Carmelo (SNICS 1036), in the National Institute for Forestry, Agriculgture and Livestock Research (*Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias*, INIFAP) at the experimental field in Huimanguillo, Tabasco, Mexico (17° 47' 07.9" N and 92° 57' 20.0" W), inside the cacao germplasm garden (INEGI, 2018). Five trees were selected and five flower buds were selected randomly without considering position on the tree (n=225 flower buds). The measurements were made with a digital Vernier (ISIZE, 0-150 mm, Mexico) on the length and diameter of the flower bud without considering the stem, between 30 and 35 days since the flower bud emerges until the opening happens. Starting with the formation of the fruit, the morphology of the fruit was evaluated every 15 days until reaching physiological maturity (Enríquez, 2004).

Morphological variables: Nine qualitative morphological variables were analyzed: Shape of the pod (SP), basal construction (BC), shape of the apex (SA), surface of the pod (S), depth of the furrows (DF), color of the pulp (CP), shape of the grain (SG), color of the cotyledon (CC), color of the fruit; and 12 quantitative variables: fruit diameter (FD), fruit length (FL), fruit length/diameter ratio (FL/FD), fruit weight (FW), exocarp thickness (ET), grain number (GN), grain weight per pod (GWP), grain length (GL), grain width (GW), grain length/width ratio (GL/GW), grain thickness (GT), average grain weight (AW), according to the International Union for the Protection of New Varieties of Plants *Theobroma cacao* L. (UPOV 2011). The information was analyzed by defining descriptive statistics, principle components analysis (PCA), and Pearson's correlation, using the statistical package Metaboanalyst 5.0. and Infostat-Profesional, version 1.1 (2020).

RESULTS AND DISCUSSION

Flower buds

Figure 1 shows the evolution of the flower bud growth until its opening with relation to the bud total length and diameter, starting for 7 days after installation (dai) and lasting until 30 to 35 days, similar to what was reported by López *et al.* (2018). Caehui obtained a record of 42 days, which was higher than the other clones, associated to intrinsic, genetic and physiological factors. The clones that present the highest total length ± standard deviation of the bud were Chak with 7.4±0.01 mm, Canek and Caehui with 7.3±0.20 mm, respectively, and the lowest total length was seen in Chibolon with 6.2±0.10 mm. The other clones remained at between 6.2 and 7.3 mm. In the variable diameter, the clones that reached the highest value were Chak with 4.2±0.20 mm, Caehui and Tabscoob, both with 4.1±0.13 mm. The clones K'in and Canek were the ones that presented the smallest diameter with 3.5±0.20 mm each, the other clones and the control were found between 3.2 and 4.2 mm. Castro *et al.* (2017) report an initial value of 4±1 mm and final value of 15±1 mm, data that are apparently higher than those reported for this study, although they consider the stem within the measurement of the flower buds.

Figure 1 does not show a sigmoidal pattern as reported by López *et al.* (2018); however, a linear behavior was seen for longitudinal growth since day 7 achieving 42% of the final size, and prolonged until day 21 with 75% of its final size. For the variable diameter, 52% was achieved in the first seven days while 77% of this variable was reached starting on day 21.

Growth in cacao fruits: Changes in fruit length and diameter (Figure 2) were described in this stage, considering the measurement unit from the moment when it reached the critical point of 15 days after the start of pollination.

The highest value in length was K'in with 252±3.54 mm and Olmeca with 220±0.70 mm, in a period of 195 days and 165 days, respectively. Meanwhile, Caehui with 103±3.74 mm presented the lowest length in 120 days. For the final diameter of the fruits with largest diameters, it was between 102±4.9 mm in Supremo with 150 days, followed by Tabscoob with 101.2±1.14 mm with 195 days.

The result of the lowest diameter at the end of development was shown by Caehui with 38.6±5.98 mm in 120 days. Figure 2 shows that the growth speed presented an inflection

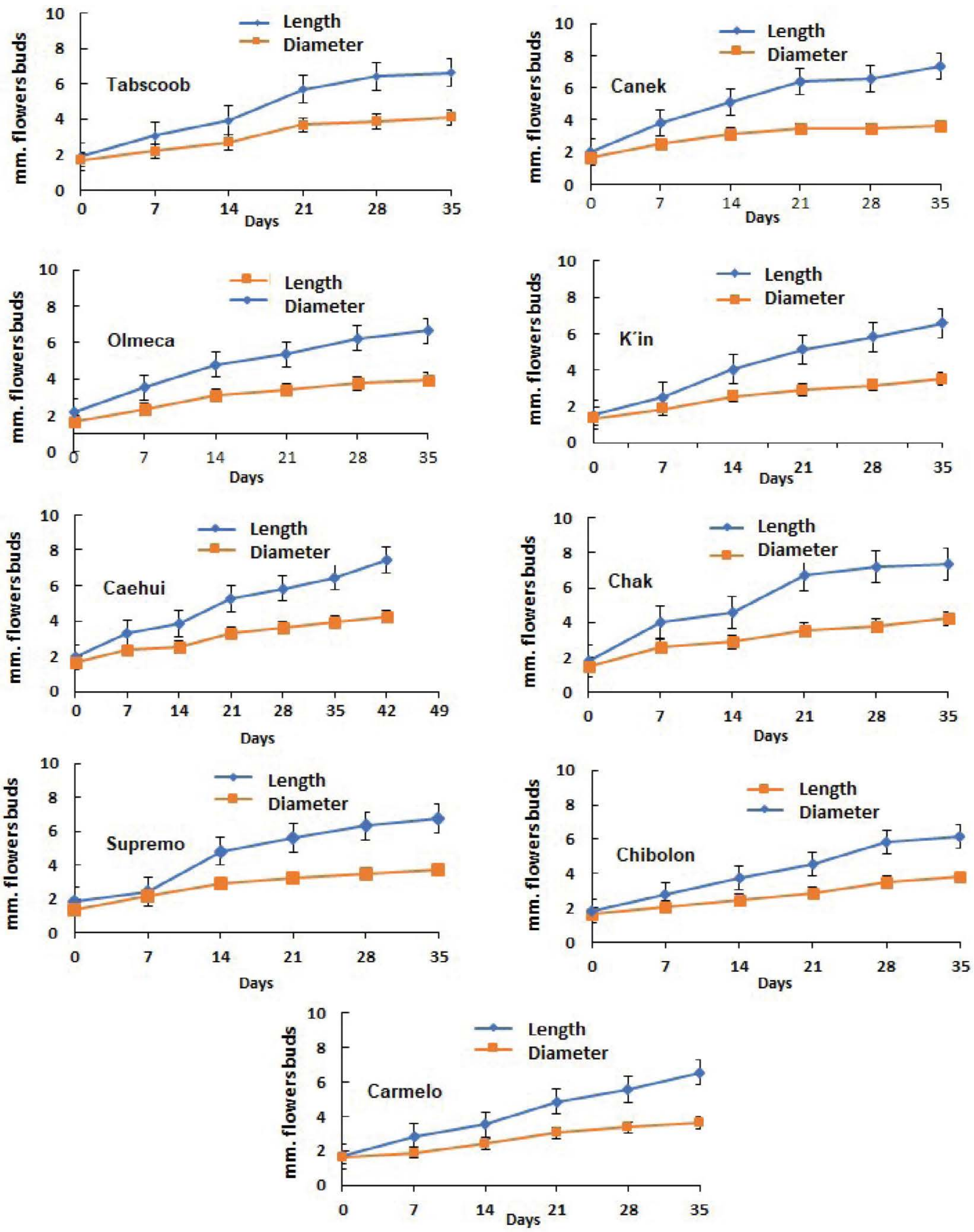

Figure 1. Evolution of phenological growth of diameter and total flower bud lenght in eight cocoa clones and Carmelo control. The mean values and the vertical line indicate ± standard error (SE).

point since day 90, with fruits in this stage reaching an increase in length and diameter of 38%, which represents the change in linear trend toward a non-linear increase. Of the fruit growth, 70% was achieved in 120 days, period at which the growth speed is slow since the cell division and elongation processes stop, causing the change in color of the fruits (López *et al.*, 2018; Enríquez, 1985). Starting at 165 days, it is the last phase of development, without apparent changes, which is associated to the maturation of the fruit.

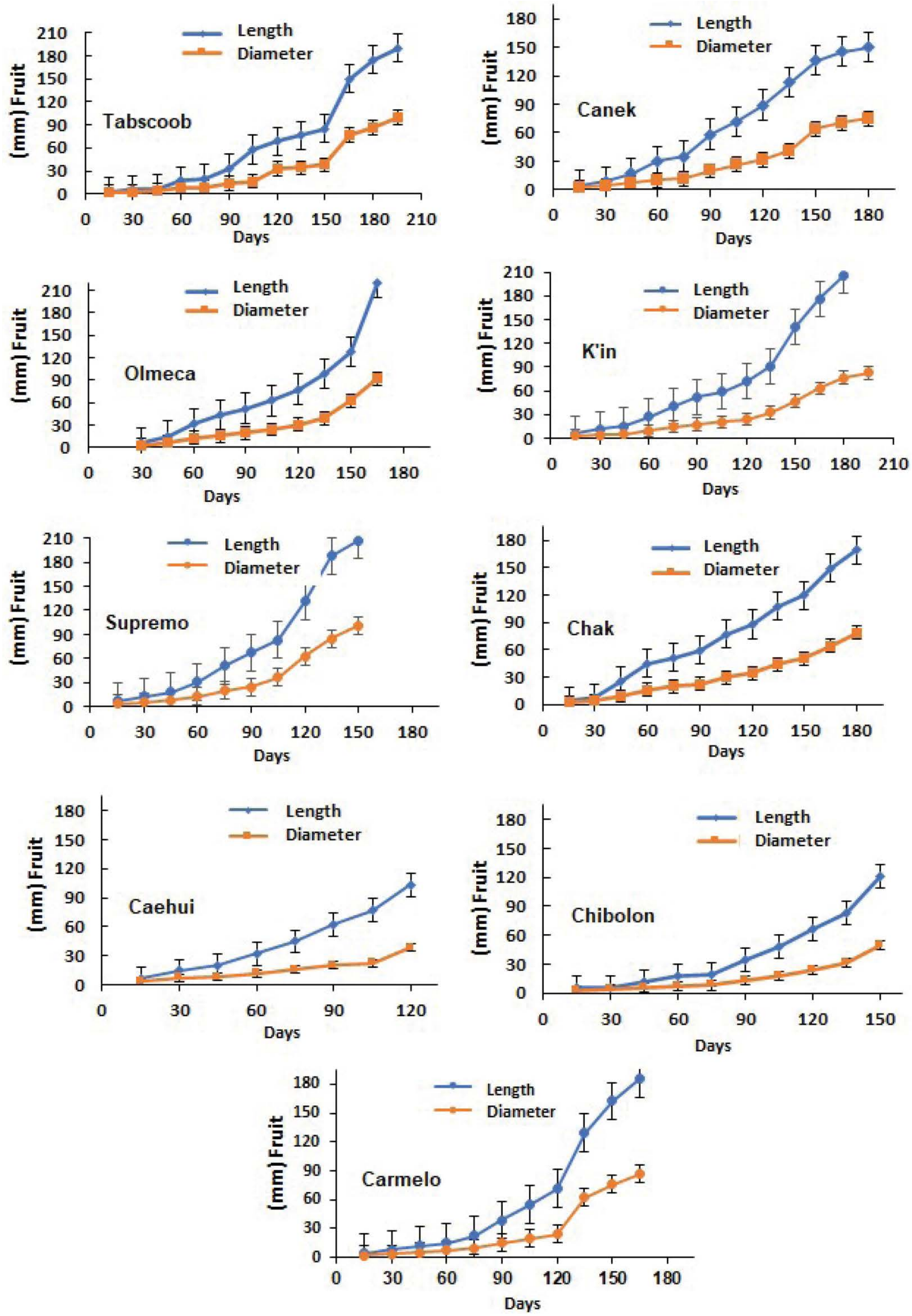

Figure 2. Evolution of phenological growth of diameter and total friut lenght in eight cocoa clones and Carmelo control. The mean values and the vertical line indicate ± standard error (SE).

Qualitative morphological characterization in the fruits

The physical characteristics in fruits of the clones were recorded in Table 1. The analysis result showed that the elliptical pods (44.4%) dominated as morphological characteristic of the fruit shape, followed by oblong (33.3%), which was similar to what was reported by Castillo (2018), who found mostly elliptical shape which refers to these characteristics being of Creole type. In the case of the basal construction, the absent state predominated with 55.5%, followed by the weak and moderate state (22.2%). Another predominant characteristic in fruits for this study was the surface, presenting smooth or slightly rough with greater frequency, with global percentage of 77.7%. In this regard, Graziani *et al.* (2002) found that sharp tips, absent basal construction and intermediate roughness predominate in the Creole-type cacao, while forastero types present melon

Table 1. Frecuency of qualitativa variables in fruits of cocoa clones.

Variables	State	% global
1) Fruit: shape	1=ovate 2=circular 3=elliptic 4=oblong 5= obovate	11.11 0 44.44 33.33 11.11
2) Fruit: basal constriction	1=absent 2=weak 3=moderate 4=strong	55.55 22.22 22.22 0
3) Fruit: shape of apex	1=waisted 2=acute 3=obtuse 4=rounded	11.11 44.44 44.44 0
4) Fruit: Surface	1=smooth or slightly 2=moderately rough 3= very rough	77.77 22.22 0
5) Fruit: Depth between ridges	1=absent or very shallow 2=shallow 3=medium 4=deep	33.33 66.66 0 0
6) Fruit: Color of pulp	1=white 2=ligth cream 3=dark cream	11.11 66.66 22.22
7) Seed: shape in longitudinal section	1=oblong 2=elliptic 3=ovate	33.33 11.11 44.44
8) Seed: cotyledon color	1=white 2=cream 2=pink 3=dark red 4=dark purple	11.11 11.11 11.11 33.33 33.33
9) Fruit: color	1=yellow 2=yellow/green 3=yellow/orange 4=orange/purple	22.22 44.44 22.22 11.11

shape, obtuse tip, without strangling and without roughness. In another context, the fruit color reached a different coloration when it was found in physiological maturity; in this case 44.4% corresponded to yellow/green tonalities, 22.22% to yellow and yellow/orange colors. In this last morphological characteristic mentioned, Bartley (2005) revealed that the red fruit color could be representative in face of the Creole group and as a result of descendants in this group having inherited this trait. In another study, Ha *et al.* (2016) have indicated the presence of orange tones for Creole cacao, while yellow tones indicate the presence of forasterio and trinitario cacao. Our study showed that the depth of the furrows corresponded to 66.6% in the variable low depth, and some studies indicate that deep furrows are characteristic of Creole cacao (Chacón *et al.*, 2011). With 66.6% clones present medium exocarp thickness, the same percentage corresponds to the variable coloring in the pulp, with light cream color standing out. The characteristics of exocarp thickness suggest the presence of hybridization by populations of the trinitario complex, a characteristic of heterogeneity in the characteristics of the fruits (Braudeau, 1970). When it comes to the color of cotyledons, violet and purple were the dominant ones (33.33%). The color has been a distinctive trait to differentiate cultivars, highlighting that white cotyledons are associated to Creole cacao (Ramos-Ospino *et al.*, 2020). The shape in longitudinal section of the seed represented 44.44% in the oval shape.

Quantitative morphological characterization in the fruits

The clones and Carmelo were compared in terms of the morphological traits of fruit and seed of eleven quantitative traits. The variable NG showed the lowest coefficient of variation (4.52%), and the variable FW presented the highest coefficient of variation (22.17%), in addition to a high mean in comparison to the other variables studied (Table 2). In a similar study, Ramírez-Guillermo *et al.* (2018) mentioned as results intervals of broad

Table 2. Statistical parameters of 12 morphological quantitative variables evaluated in cocoa clones and Carmelo control.

Variables	Means	SD	Min.	Max.	C.V.
Fruit diameter (mm)	85.14	4.93	77.10	90.00	5.79
Fruit length (mm)	168.70	15.93	146.43	196.00	9.44
Fruit length/diameter ratio	2.03	0.23	1.63	2.35	11.19
Fruit weight (g)	574.45	127.37	403.63	840.59	22.17
Fruit exocarp thickness (mm)	13.62	1.37	11.16	15.00	10.06
Fruit number seeds	40.45	1.83	37.52	42.95	4.52
Seed weight per fruit (g)	140.75	25.78	108.27	191.07	18.32
Seed length (mm)	23.16	1.68	19.84	25.57	7.28
Seed width (mm)	13.03	1.16	10.99	14.44	8.90
Seed length/width ratio	1.79	0.09	1.66	1.99	5.09
Seed thicknees (mm)	8.51	0.50	7.51	9.20	5.88
Average seed weight	1.41	0.37	0.66	2.63	0.26

SD=Standard Deviation; CV=Coefficient of Variation (%).

variation, especially for FW. The length/diameter ratio in grain as in fruit presented lower values in terms of mean, deviation, minimums, and maximums.

Correlation of the morphological variables in cacao fruits

Table 3 shows the positive and negative correlations with significant character ($p<0.05$ and $p<0.001$). The fruit weight (FW) showed a significant positive correlation ($p<0.05$ and $p<0.001$) with most of the morphological variables with the exception of the (NG), (GL/GD) and (GT). One of the most important aspects that was found is the highly significant positive relation ($p<0.001$) between (FW) and (GW). At the same time, grain weight (GW) showed that only two variables were not significant (NG and GT) and the others showed a positive correlation ($p<0.05$ and $p<0.001$), with the exception of the GL/GD ratio which was negative. In a study conducted by Vázquez and García (2005), in a cacao germplasm bank with 20 cacao genotypes of different genetic origins found that the coefficient of correlation between the pod weight and the number of seeds was non-significant; while the correlation between the weight of the pod and the fresh weight of the seed was positive and highly significant, which agrees with our results.

Principal Components Analysis

The principal components analysis (PCA) revealed that the first axis of the CP1 is composed mainly by 37.6% of the variation, showing it is composed primarily by the geometrical traits both of the fruit and of the cotyledon: length, diameter, fruit weight and grain weight per pod correlated positively. The second axis of the CP2 represented 24.6% of the total variation, highlighting some qualitative traits, among them: surface, fruit color, shape of the cotyledon, fruit and tip, and basal construction. Figure 3 shows the relative

Table 3. Correlation coefficients of morphological variables of eight cocoa clones and the Carmelo control from INIFAP-Huimanguillo germoplasm garden.

	NG	GL/GW	FL/FD	GT (mm)	GL (mm)	GW (mm)	AW (g)	FD (mm)	ET (mm)	GWP (g)	FL (mm)	FW (g)
NG	1											
GL/GW	−0.0370	1										
FL/FD	−0.0616	−0.2430**	1									
GT (mm)	−0.1009	−0.0720	0.0277	1								
GL (mm)	−0.1274	0.3586**	−0.0868	0.0188	1							
GW (mm)	−0.0908	−0.4690**	0.1204	0.0548	0.6502**	1						
AW (g)	−0.0746	−0.1731*	0.1924**	0.1196	0.3081*	0.4437**	1					
FD (mm)	0.0645	−0.0129	−0.4216**	0.0306	0.1839*	0.1846*	0.1382*	1				
ET (mm)	−0.0629	−0.1464*	−0.0558	0.0641	0.1732**	0.2854**	0.2061*	0.5083**	1			
GWP (g)	0.1331	−0.2856**	0.1825*	0.0943	0.1620*	0.3853**	0.3592**	0.2847**	0.2809**	1		
FL (mm)	−0.0463	−0.2639**	0.6108**	0.0829	0.0267	0.2388**	0.3197**	0.4056**	0.3065**	0.4213**	1	
FW (g)	0.0605	−0.1276	0.2137*	0.0606	0.1829*	0.2901**	0.3364**	0.5029**	0.5246**	0.3787**	0.5798**	1

NG: Grain number; GL/GW: grain length/width ratio; FL/FD: Fruit length/diameter ratio; GT: grain thickness; GL: grain length; GW: grain width; AW: average grain weight; FD: fruit diameter; ET: exocarp thickness; GWP: grain weight per pod; FL: fruit length; FW: fruit weight. * ($p<0.05$), ** ($p<0.001$).

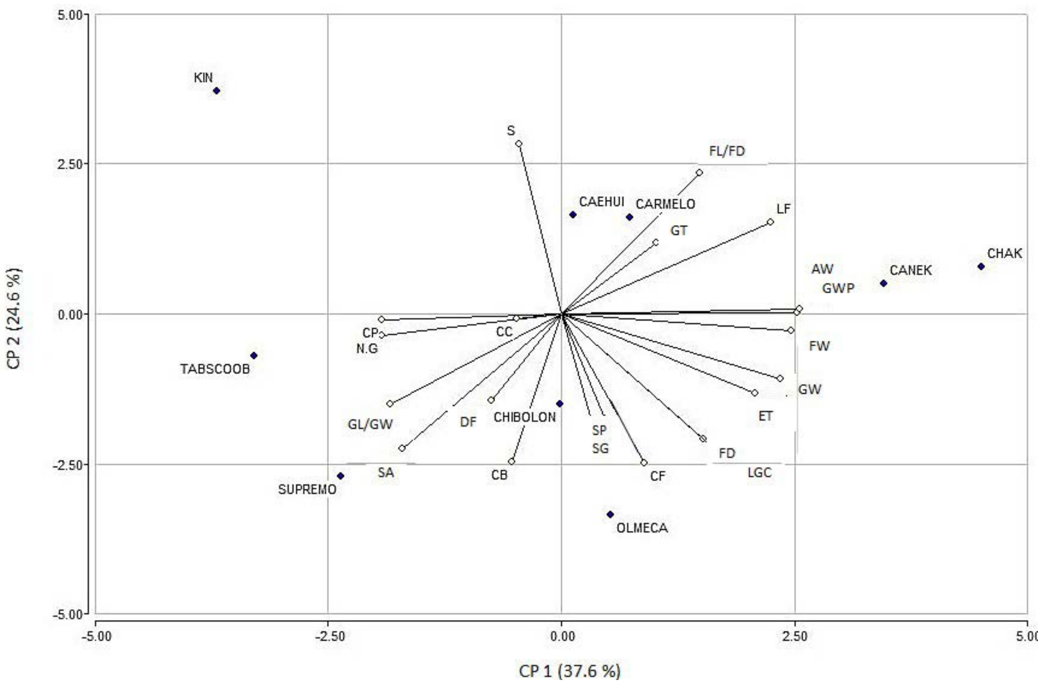

Figure 3. Dispersion of qualitative and quantitative morphological variables on the principal component axes (CP1 and CP2) accounting for 62.5% of the total variation. NG: grain number; GL/GW: grain length/width ratio; FL/FD: fruit length/diameter ratio; GT: grain thickness; GL: grain length; GW: grain width; AW: average grain weight; FD: fruit diameter; ET: exocarp thickness; GWP: grain weight per pod; FL: fruit length; FW: fruit weight; SP: Shape of the pod; BC: basal constriction; SA: shape of the apex; S: surface of the pod; DF: depth of the furrows; CF: color of the fruit; CP: color of the pulp; CC: color of the cotyledon; SG: shape of the grain

association between variables, in function of the principal coordinates that accounted for 62.5% of the total variation.

CONCLUSIONS

The differences in measurements (length and diameter) of the flower buds are attributable to the different genetic constitution of the clones; however, knowing the opening times of these allows having an exact reference in production levels. The first two principal components (PC) in the morphological analysis described 62.5% of the total variation. The parameters that contributed most to the variability observed were FW, FD, GL, GD, ET and GWP. The study shows that the clones presented morphological variation for the characteristics of the fruit, and with that it would be expected that the physicochemical characteristics give different quality characteristics. The study revealed specific characteristics of the clones that can be used as selection criteria to improve the yield and quality indicators.

ACKNOWLEDGEMENTS

The authors thank the Cacao Regulating Council through the Fomix - CONACYT project (TAB-2018-01-01-84312), as well as the Mexican National Council of Science and Technology for the support through scholarship number 882616.

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