Morphological characterization of cacao (*Theobroma cacao* L.) criollo type in Mexico

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ABSTRACT
Objective: Achieve the morphological characterization of Criollo-type cocoa from Mexico.
Design/methodology/approach: For morphological characterization, 17 Criollo-type cocoa accessions and 30 varietal descriptors were proposed by Avendaño *et al.*, (2014), and this was carried out on five-year-old trees in the Rosario Izapa Experimental Field of INIFAP during two production cycles.
Results: With the first three principal components, 47.3% of the variation was explained and the variables that most explained this variation were the color of the young leaf, anthocyanin pigmentation of the pedicel, basal constriction of the fruit, shape of the fruit apex and length/diameter ratio of the fruit; sepal length, sepal width length, fruit length, fruit exocarp thickness, seed width, seed length/diameter ratio and cotyledon color. The cluster analysis allowed us to differentiate two groups where the color of the unripe fruit was one of the descriptors that contributed the most to forming the groups.
Limitations on study/implications: Knowledge of the diversity of Mexican Criollo-type cocoas allows for establishing strategies for conserving and using this type of cocoa.
Findings/conclusions: In Mexico, the Criollo cacao genetic group presents a wide morphological variation in the descriptors of leaf, flower, fruit and seed. The shape of the apex of the leaf, the anthocyanin pigmentation in the flower, the color in the mature and immature state, and the shape and basal constriction of the fruit, as well as the color of the cotyledon, are the descriptors that allowed us to differentiate the Criollo cocoas studied.

Keywords: Criollo cocoa, diversity, characterization.

INTRODUCTION

In Mexico, the cultivation of cocoa (*Theobroma cacao* L.) is of great cultural, economic, social and environmental importance since most of the area planted in Mexico is under the agroforestry system (Barómetro del cacao, 2022). Mexico ranks 12th worldwide in
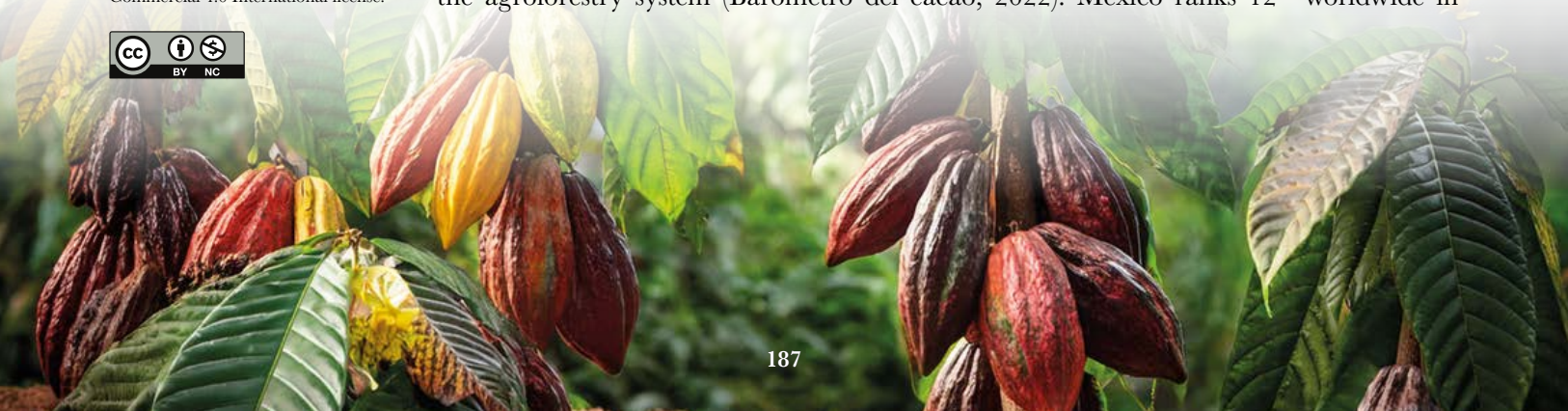
production, accounting for approximately 0.8% (ICCO, 2024) and currently, around 52,449 ha are cultivated, with a production of 28,119 tons and a production value of $1,158,661 (SIAP, 2024). In Mexico, three genetic types of cocoa are recognized: forastero (*T. cacao*, subs. *sphaerocarpum*), criollo (*T. cacao* subs. *cacao*) and the cross between these, trinitario (Lachenaud & Motamayor, 2017); each with its particular morphological characteristics (Cuatrecasas, 1964) and with different varieties within each genetic group.

For their part, Avendaño *et al.*, (2011) mention that in Mexico, the three genetic groups of cocoa are cultivated with different varieties, with Forastero and Trinitario predominating and, to a lesser extent, Criollo type cocoas, even though the international demand for Criollo and Fine Aroma types has grown (Barómetro del cacao, 2022). In addition, Avendaño *et al.* (2018) disseminate Criollo-type cocoas and describe the morphology of the fruits of some Criollo cocoas from Mexico. The fruits of the Forastero-type cocoa are melon-shaped, have a hard and woody shell, are smooth to medium roughness, are flattened beans, and are purple. In Criollo types, the fruits are elongated and green and reddish, with roughness, absent or light basal constriction and rounded white seed color, and these have a higher quality than Forastero and Trinitario cocoa (Avendaño *et al.*, 2018). On the other hand, Trinitario cocoas have fruits with variable roughness, thin to medium shell, the seed is pink to purple, and the flavor and aroma are delicate to medium. In the different cocoa-growing areas of the world and Mexico, different types of characterization have been carried out, highlighting the morphological, biochemical and molecular to know the genetic diversity of the different varieties of the cocoa present, focusing mainly on the fruit and the seed; however, it is necessary to know the morphological diversity of the plant, leaf, flower, fruit and seed (Avendaño *et al.*, 2014), to correlate specific morphological markers of the leaves, with the flower and finally with the seed, the morphological characterization of Criollo cocoas was carried out, to differentiate the Criollo type varieties, where Mexico is characterized by presenting a wide genetic diversity.

MATERIALS AND METHODS

Plant material: 17 criollo cocoa genotypes were used, which are kept in the Criollo Cocoa Germplasm Bank of the Rosario Izapa Experimental Field of INIFAP (Table 1).

Morphological characterization: For the morphological characterization of the plant, leaf, flower, fruit and seed of the 17 Criollo cocoa genotypes, 30 varietal descriptors proposed by Avendaño *et al.*, (2014) were used.

Statistical analysis: The data for each descriptor were captured and systematized in an Excel spreadsheet, and then a principal component analysis was applied to them in order to detect through the new variables (principal components) which descriptors are differentiating the criollo cocoas, and finally, a hierarchical cluster analysis was applied to observe the grouping of the different genotypes; for this purpose, the SAS statistical package ver. 9.0 was used.The morphological characterization was conducted in the unique setting of the Rosario Izapa Experimental Field, a location known for its conducive conditions for cocoa cultivation. The study spanned two production cycles, focusing on five-year-old trees, with the aim of verifying the stability of the evaluated characters.

Table 1. Criollo cocoa genotypes used in the morphological characterization.

Number	Genotype	Acronym	Origin
1	Verde Gustavo	VerGus	Chiapas
2	Rojo Gustavo	RojGus	Chiapas
3	Rojo Samuel	RojSam	Chiapas
4	Baalam	Baa	Chiapas
5	Loxicha	Loxicha	Oaxaca
6	Real Soconusco	RealSoc	Chiapas
7	Real Soconusco 2	RealSoc2	Chiapas
8	Tazantán 1	Tuz01	Chiapas
9	Tuzantán 2	Tuz02	Chiapas
10	Tuzantán 3	Tuz03	Chiapas
11	Cuyul	Cuyul	Yucatán
12	Ces-6	Ces06	Centro América
13	Real Soconusco 3	RealSoc3	Chiapas
14	Lagartito Rojo	Ltorojo	Chiapas
15	Carmelo	Carmel	Tabasco
16	Lacandón	Lacando	Chiapas
17	Sak- Balam	Sakbal	Chiapas

RESULTS AND DISCUSSION

According to the principal component analysis (PCA), the first four principal components (PC) explained 56.2% of the variation; PC1 explained 21.2%; PC2 14.8%; PC3 10.3% and PC4 9.9% (Table 2). This is a significant finding. It is interesting to note that Osorio-Guarin *et al.* (2017), when characterizing 141 cacao accessions with 18 qualitative characteristics, found that the first five principal components explained 60.6% of the variation. In our study, the first five principal components explained an even higher 63.4% of the variation, providing a valuable comparison point for our research community.

The variables that contributed the most in each PC were: for PC1, Sepal Length (SEL); Fruit Shape (FS), Fruit Basal Constriction (FBC), Fruit Apex Shape (FAS), Fruit Length (FL), Fruit Length/Diameter Ratio (FLDR), Green Fruit Color (GFC), =Exocarp Thickness (ET); Fruit Pulp Sweetness (FPS), Cotyledon Color (CC); PC2, Anthocyanin

Table 2. Eigenvalues of the correlation matrix for 30 varietal descriptors of 17 Criollo-type cocoa genotypes.

Eigenvalues of the correlation matrix				
PC	Eigenvalues	Difference	Proportion	Cumulative
1	6.582	1.985	0.212	0.212
2	4.597	1.411	0.148	0.361
3	3.186	0.118	0.103	0.463
4	3.067	0.739	0.099	0.562
5	2.328	0.143	0.075	0.637

PC=Principal component.

Pigmentation of the Pedicel (APP), =Sepal width Length (SLA), Ligule color (LC), Seed Length/Diameter ratio (SLD), =Seed Thickness (ST); for PC3, Anthocyanin Pigmentation of the Sepal (APS), Fruit Pulp Color (FPC), Seed Length (SL); =Seed Width (SW) (Table 3). Osorio-Guarin *et al.* (2017) report that the variables that contributed most to differences in cocoa accessions were leaf bud color, shape and basal constriction of the fruit, thickness of the exocarp, and anthocyanin pigmentation of the pedicel. In addition, Bidot *et al.* (2017) reported that, when using 33 descriptors to characterize cacao varieties in Cuba, the ones that contributed the most in the seed were flat cross section and the intensity of the violet color; in the fruit: color, depth of the grooves, roughness and hardness of the mesocarp; and in flower: anthocyanin pigmentation of the staminodes and the peduncle and the color of the ligule.

Principal component analysis showed a wide dispersion in Criollo-type cocoa from Mexico. Varietal descriptors of flower, fruit and seed mainly give this dispersion. Vásquez-García *et al.* (2022), when characterizing different varieties of cocoa using 20 descriptors, found that leaf descriptors explained 36.07% of the variation, flower descriptors 20.54%, fruit 19.84% and seed 30.74%. On the other hand, Rangel-Fajardo *et al.* (2012) mention the importance of the embryonic sac and seed development based on the size of the fruit in the Criollo cocoa variety "Carmelo." Even though the environment highly influences the fruit size descriptor, it does allow differentiation of the genetic types of cocoa.

When graphing components 1 and 2, it can be observed that the dispersion is not random; this is because the Criollo type cocoas with red fruits in an immature state and wrinkled such as Baalam, red lizard, real soconusco 3, red Gustavo, real soconusco, real soconusco 2 and red Samuel; showed a tendency to come together and share other descriptors such as redbud color, red sepal color and larger flower size, without the basal constriction of the fruit and the shape of the fruit; in addition, they showed a more significant influence in the separation of red fruits with those of green color in an immature state (Figure 1). On the other hand, Marcano *et al.* (2008) report that red pigmentation in the different organs of cocoa (leaf, flower, and fruit) allows the differentiation of the varieties. They also found a high association with QTL markers. On the other hand, for Criollo-type cocoas with green color in an immature state, such as Lacandón, Carmelo, CES-06, Tuzantan 02 and Sak Balam, the descriptors that had the most influence were those of the seed (thickness, width and length) and in the fruit the shape, diameter and shape of the apex (Figure 1).

Figure 2 shows the dispersion of Criollo-type cocoas based on CP1 and CP3; there is a similar trend to Figure 1, where the seed descriptors (length and width) and shape of the fruit apex unite the green-colored cocoas in an immature state and the flower and fruit descriptors for the cocoas with red-colored fruits in an immature state (Figure 2).

According to the cluster analysis, two groups were formed, where the color of the immature fruit was the one that separated the groups; in Group I, the green ones were grouped, and in Group 2, the red ones mainly were grouped, except the green Gustavo. In addition, the GI is characterized by having criollo cocoas where the cream cotyledon color predominates, thus suggesting fine aroma cocoas (Figure 3).

Table 3. Eigenvalues and Pearson correlation coefficient for 30 varietal descriptors of 17 Criollo-type cacao genotypes.

Descriptor	PC1	PC2	PC3	Pearson Correlation Coefficient		
				PC1	PC2	PC3
SLB	−0.11	0.02	0.02	−0.28	0.03	0.04
SBLB	−0.11	−0.10	0.01	−0.27	−0.21	0.02
IGCLB	0.15	0.07	0.10	0.39	0.16	0.19
SALB	0.08	−0.08	0.18	0.21	−0.18	0.33
CYL	0.30	0.21	0.09	**0.76***	0.46	0.17
APP	0.17	0.28	−0.09	0.44	**0.60***	−0.16
SEL	0.23	−0.22	0.14	**0.58***	−0.47	0.25
SLA	0.19	−0.31	0.17	0.48	**−0.65***	0.31
APS	0.02	0.04	−0.40	0.04	0.09	**−0.72***
LC	0.01	0.24	0.13	0.03	**0.51***	0.22
APS	0.15	0.03	−0.11	0.37	0.07	−0.19
FS	0.27	0.08	0.01	**0.68***	0.17	0.03
FBC	0.29	0.06	0.16	**0.74***	0.12	0.28
FAS	−0.21	−0.26	−0.11	**−0.55***	−0.55	−0.19
FL	0.26	−0.11	0.03	**0.68***	−0.23	0.05
DF	−0.17	−0.19	−0.03	−0.44	−0.40	−0.05
FLDR	−0.32	−0.07	−0.02	**−0.83***	−0.16	−0.04
SF	0.09	0.21	0.14	0.24	0.46	0.25
DGF	0.02	0.02	0.20	0.06	0.04	0.37
CF	0.08	0.11	−0.27	0.21	0.24	−0.48
GFC	0.21	0.19	−0.20	**0.53**	0.41	−0.37
ET	0.21	−0.16	−0.04	**0.53**	−0.34	−0.08
FPC	0.02	0.10	−0.36	0.05	0.21	**−0.63***
FPS	0.20	−0.07	0.15	**0.51**	−0.14	0.26
TNS	0.04	−0.20	0.06	0.09	−0.44	0.10
FS	0.05	0.21	0.01	0.14	0.46	0.01
SL	−0.20	0.06	0.38	−0.51	0.13	**0.69***
SW	−0.20	0.25	0.32	−0.52	0.53	**0.56***
SLD	0.07	−0.35	0.10	0.19	**−0.74***	0.17
ST	−0.12	0.28	0.26	−0.31	**0.60***	0.47
CC	0.27	−0.20	0.09	**0.69***	−0.42	0.15

PC=Principal Component; SLB=Size of the Leaf Blade; SBLB=Shape of the Base of the Leaf Blade; IGCLB=Intensity of the Green Color of the Leaf Blade; SALB=Shape of the Apex of the Leaf Blade; CYL=Color of the Young Leaf; APP=Anthocyanin Pigmentation of the Pedicel SEL=Length of the sepal; LAS=Length of the width of the sepal; PAS=Anthocyanin pigmentation of the sepal; CL=Color of the ligule; APS=Anthocyanin Pigmentation of the Staminode; FF=Shape of the fruit; CBF=Basal constriction of the fruit; FAF=Shape of the apex of the fruit; LF=Length of the fruit; DF=Diameter of the Fruit; FRLD=Length/diameter ratio of the fruit; SF=Surface of the fruit; DGF=Depth of the Groove of the Fruit; CF=Color of the fruit; CFV=Color of the green fruit; GEF=Exocarp thickness; CPF=Fruit pulp color; DPF=Fruit pulp sweetness; TNS=Total number of seeds; FS=Seed shape; LS=Seed length; AS=Seed width; RLD=Seed length/diameter ratio; GS=Seed thickness; CC=Cotyledon color.
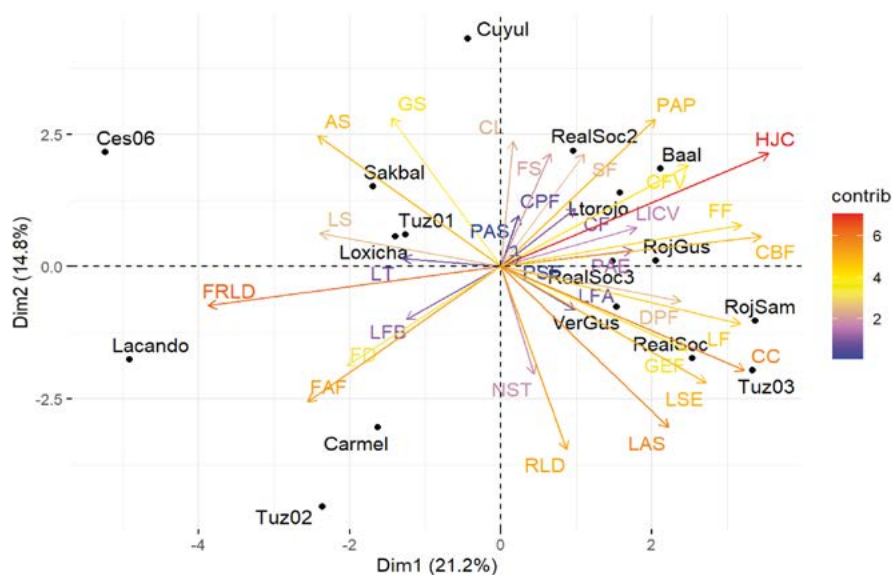
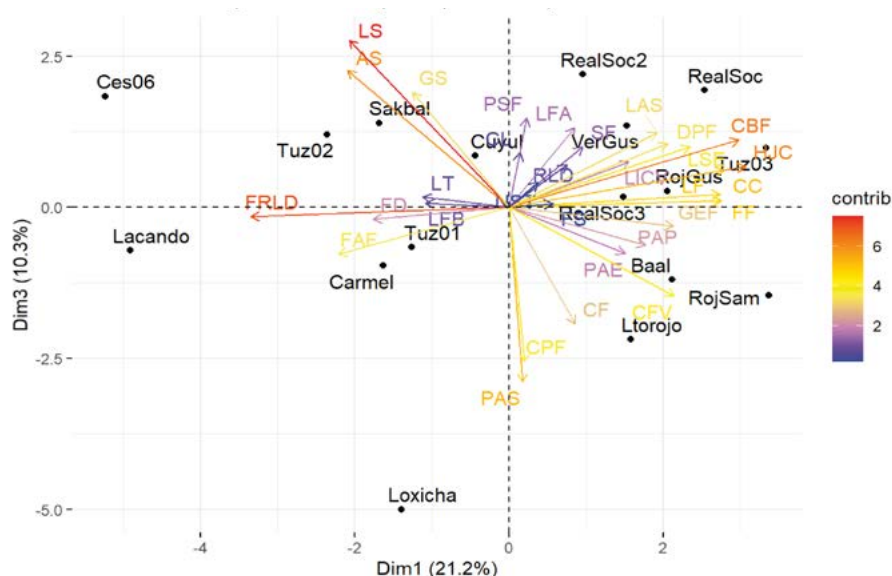**Figure 1**. Dispersion of 17 Criollo-type cocoa as a function of principal components 1 and 2.

Figure 2. Dispersion of 17 Criollo-type cocoa as a function of principal components 1 and 3.

The morphological characterization of the leaf, flower, fruit and seed allowed us to differentiate the two groups formed in the hierarchical cluster analysis. In bud color, the brown color predominated in GI, and in GII, the colors ranged from medium red to dark red (Figure 4). In the flower, the groups were differentiated by the anthocyanin pigmentation of the pedicel and the sepal; in GII, the pedicels presented more excellent anthocyanin pigmentation (Figure 4). In fruit, the groups presented marked differences; in GI, the ovate shape predominated, yellow color, the apex was obtuse to acute and weak
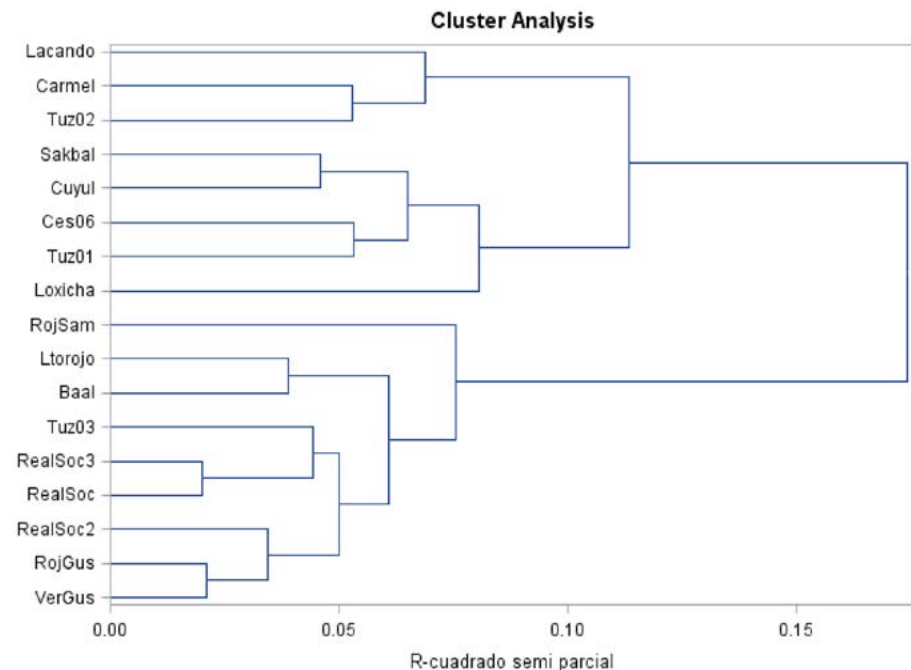
Figure 3. Dendrogram of 17 Criollo-type cocoa based on 30 morphological varietal descriptors.

basal constriction, and in GII, the shape was obovate, red color, notched apex and strong basal constriction (Figure 5). The seed also presented differences between groups; in GI, the cream color and ovate shape predominated (Figure 5). Finally, in the leaf descriptors, the shape of the apex allowed the differentiation of the groups; in the GI, the shape of the acute apex predominated (Figure 5). The diversity of bud colors, color and length of sepal, ligule and pedicel suggests that during the domestication process and given that this is dynamic, natural crossings continue to occur, which favors the appearance of new forms and colors of bud and flower (Figure 4).

The same occurs with the shapes, colors and sizes of leaves, fruits and seeds (Figure 5). Natural crossbreeding, environmental pressure and the selection that producers have been carrying out for many years have allowed the emergence of new varieties of Criollo cocoa, which can be a fundamental source for genetic improvement programs or for the selection of new aromas and flavors that the national and international market is demanding. The development of the chocolate flavor is highly dependent on multiple factors, including the conditions where the cocoa is grown, for example, the soil, climate and the genetics of the cocoa (Engeseth & Ac Pangan, 2018; Vázquez-Ovando *et al.*, 2015). In addition to the morphological differences between the genetic groups of cocoa, there are also marked differences in volatiles, as reported by Xiao-Wei *et al.* (2017). The highest concentration of volatiles is reported in the Trinitario group (79.7%) and the lowest in the Forastero group (70.39%). Also, Vera *et al.* (2014) report that when characterizing cocoa clones from Ecuador, the physical and chemical variables are associated, as well as the sensory attributes of cocoa, such as fruity, sweet, and floral.
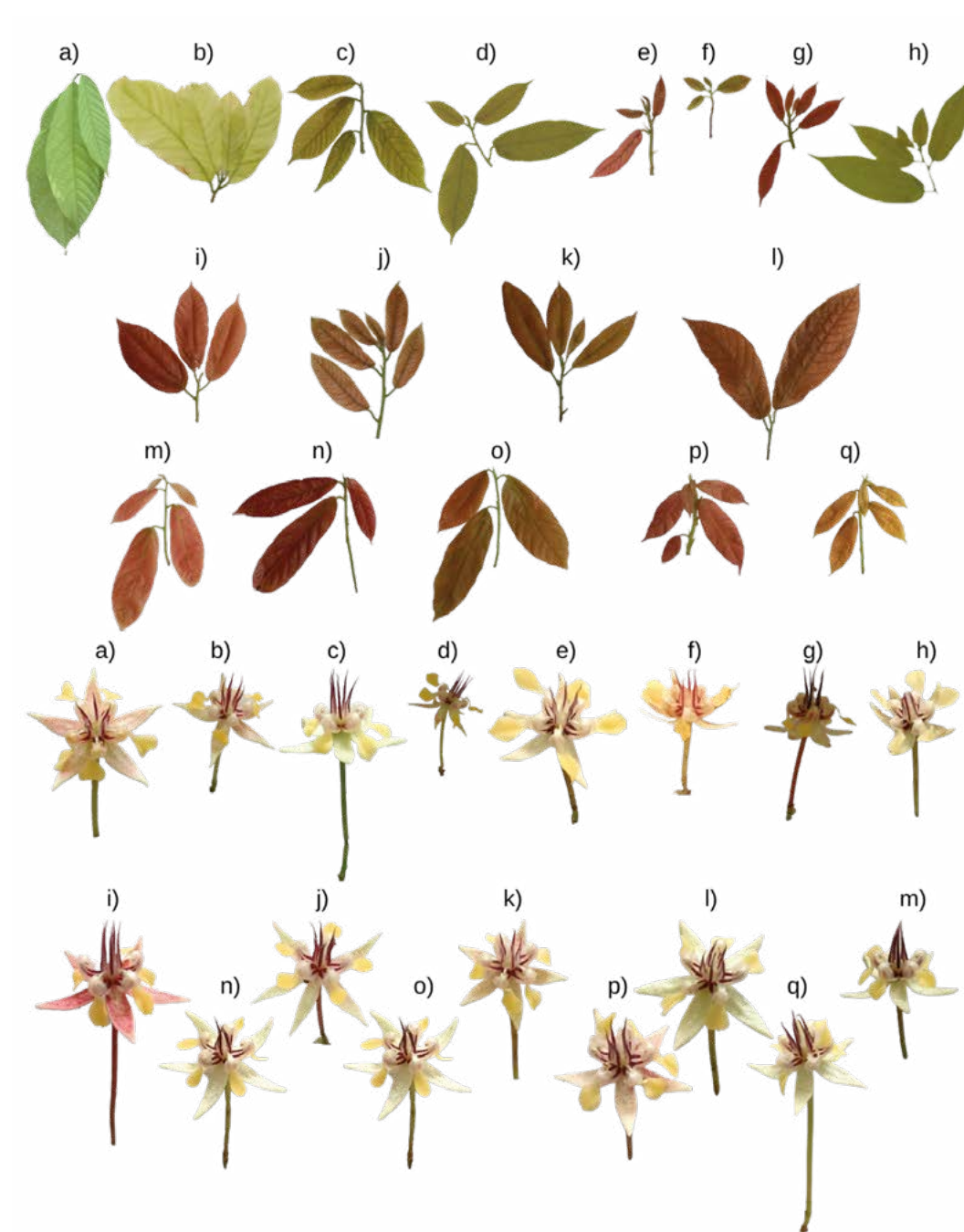
Figure 4. a) Lacando, b) Carmel, c) Tuz02, d) Sakbal, e) Cuyul, f) Ces06, g) Tuz01, h) Loxicha, i) RojSam, j) Ltorojo, k) Baal, l) Tuz03, m) RealSoc3, n) RealSoc, o) RealSoc2, p) RojGus, q) SeeGus.
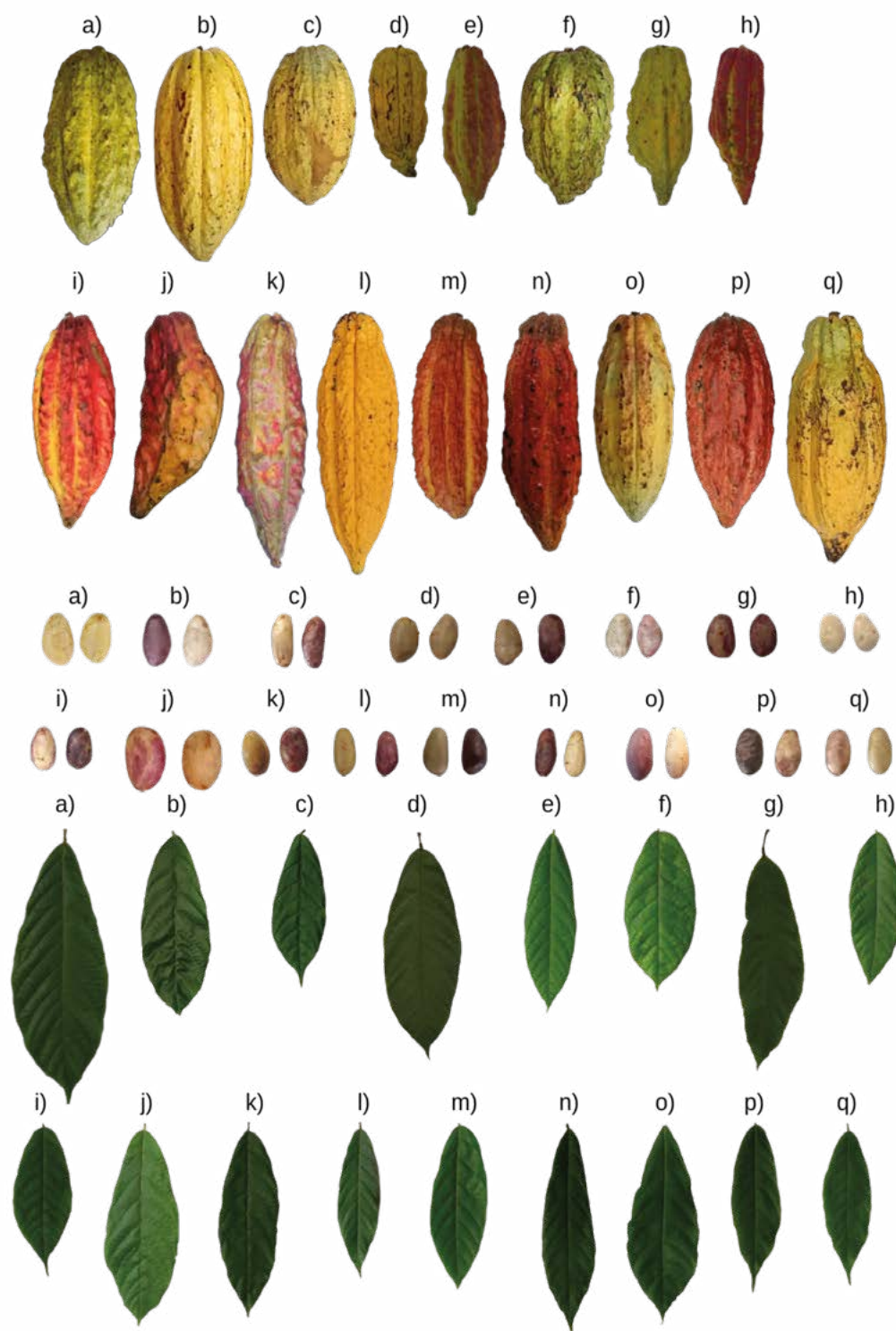
Figure 5. a) Lacando, b) Carmel, c) Tuz02, d) Sakbal, e) Cuyul, f) Ces06, g) Tuz01, h) Loxicha, i) RojSam, j) Ltorojo, k) Baal, l) Tuz03 , m) RealSoc3, n) RealSoc, o) RealSoc2, p) RojGus, q) SeeGus.

CONCLUSIONS

In Mexico, the genetic group of Criollo cacao presents a wide morphological variation in the descriptors of leaf, flower, fruit and seed. The shape of the apex of the leaf and fruit, the anthocyanin pigmentation in the flower, the color in the mature and immature state, the shape and basal constriction of the fruit, as well as the color of the cotyledon, are the descriptors that allowed the Criollo cacaos studied to be differentiated.

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