

Research Article

Phenotypic Characterization of Fine-Aroma Cocoa from Northeastern Peru

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The phenotypic characterization of cacao (*Theobroma cacao* L.) plays an important role in the generation of information for the conservation of cacao germplasm. The objective of this study is to characterize phenotypically 146 ecotypes of fine-aroma native cacao (FFNC) from northeastern Peru that were collected from 280 to 1265 metres above sea level. Morphological descriptors of fruits and seeds, sensory characteristics, and productivity descriptors were used. The data obtained were analyzed using descriptive statistics with pie charts, distribution histograms, and multiple correspondence analysis. The results showed that 76.7% of the cocoa ecotypes had green immature fruits, 73% showed slight roughness on the surface of the fruit, 54% showed an intermediate thickness of the fruit wall, and 90% had the appearance of pairs of equidistant ridges. Regarding seed characteristics, 71% showed purplish cotyledons, with a high presence of floral and fruity notes and low levels of bitterness and astringency. Likewise, 52% of the fruits and 64% of the seeds were long. More importantly, cocoa beans needed to produce between 14 and 16 pods to obtain one kilogram of dry cocoa, which reflects a good level of productivity. Finally, there was a positive relationship between elevation levels and the presence of fine-flavoured native cocoa, i.e., the greatest diversity of native cocoa with floral and fruity notes was found above 501 metres above sea level.

1. Introduction

The morphological study of plants is not only used to investigate gross aspects but also to explore and compare microscopic aspects of form, structure, and reproduction, i.e., aspects that are recognized by an observer without scientific training [1]. The characterization of species, families, and genera of plants and their morphological characters has been widely used and is becoming a useful and indispensable tool to carry out numerous studies in population genetics and agriculture [2]. Therefore, the characterization of cocoa accessions and/ or clones consists of determining the expression of traits with very high heritability, ranging from morphological characters to seed proteins and possibly including molecular markers that eliminate duplicate clones [3]. Genetic progress of cocoa (*Theobroma cacao* L.) is limited by inheritance of complex traits and the prevalence of technical problems due to mislabeling of individuals [4]. Morphological traits are used to study genetic variability, identify plants, and conserve genetic resources [5]. From the point of view of breeding, information on morphological and agronomic traits is irreplaceable [6], and the first step to consider in genetic improvement programs is to measure the genetic variability of a collection based on the use of defined descriptors.

Several authors have proposed different morphological descriptors for the identification and evaluation of the cocoa germplasm; in 2000, 65 descriptors were proposed that have been used for decades in different research centers, such as the Tropical Agricultural Research and Higher Education Center (CATIE), International Cocoa Genebank, Trinidad (ICGT), and the International Cocoa Germplasm Database (ICGD) [7]. Years later, 51 morphological descriptors were proposed, 8 for leaves, 22 for flowers, 15 for fruits, and 6 for seeds, to characterize cocoa [8]. Additionally, when conducting a characterization study of 73 cultivars from the main cocoa-producing areas in Peru, 17 morphological descriptors of flowers, fruits, and seeds were used along with 8 descriptors of agronomic and phytosanitary productivity [9]. Therefore, the use of various parameters, both morphological and phytopathological, allows the accumulation of important information that facilitates the phenotypic distinction of different trees, which will allow for outstanding materials to be included in genetic improvement programs [2].

On the contrary, the Amazon region of Peru is recognized for producing a diverse range of cocoa beans with distinctive sensory attributes (flavor and aroma) that are very different from those of other cocoa production centers, which is why this region was granted the designation of origin "Cacao Amazonas Peru." [10, 11] However, although cocoa, as well as coffee, cultivation is the most representative product of the Amazon region and can be very promising for increasing the sustainable development of cocoa activity, phenotypic characteristics of cocoa have not yet been recorded in terms of key descriptors of fruits, seeds, productivity, and sensory properties. In this sense, this research aims to perform the phenotypic characterization of 146 ecotypes of fine-aroma native cocoa from the northeastern zone of Peru.

2. Materials and Methods

2.1. Study Area. This research was carried out with 146 native fine-aroma cacao ecotypes, and the samples were harvested between the months of April and February, 2019, from the northeastern zone of Peru, which includes three main regions (Amazonas, Cajamarca, and San Martin) which were previously identified and selected; consequently, the ecotypes selected were from cacao-producing areas at elevations from 280 to 1265 metres above sea level (Figure 1).

2.2. Characterization of FFNC Ecotypes Using Morphological Descriptors. Fruit of each selected cacao plant was collected and taken to the Laboratorio de Fisiología y Biotecnología Vegetal (FISIOVEG) of the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES) of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM); the tasting room consists of a central porcelain table of 5.00 m long by 1.50 m wide, with chairs at each end to be used by the tasters, with the necessary equipment for the preservation of samples. The phenotypic characterization was carried out using a list of qualitative and quantitative descriptors for cacao, as described by Garcia-Carrión and Fernando [9]. Due to the variability in fruit and seed colors of the ecotypes in the northeastern zone of Peru, green, pigmented green, red, and pigmented red were considered as indicators of immature fruits, and white, creamy white, pinkish white, violet, and purple were considered as indicators of cotyledon color. Under these criteria, the phenotypic characterization of fine-aroma native cocoa was subject to the descriptors detailed below.

2.2.1. Morphological Descriptors of Cocoa Fruits and Seeds. We considered the criteria presented in Table 1 for the morphological description of cocoa pods and seeds.

2.2.2. Productivity Descriptors. We used the criteria described in Table 2 for the productivity descriptors.

2.2.3. Sensory Descriptors of Fresh Fruits. Five women tasters with an average age of 27, trained and accredited by the Peruvian Association of Cocoa Producers (APPCACAO) and the National System of Evaluation, Accreditation and Certification of Educational Quality (SINEACE), were consulted in order to identify the basic flavours and to evaluate the sensory characteristics of the fresh cocoa fruit. These specialists, who are researchers from the Research Institute for Sustainable Development, de Ceja de Selva of the National University Toribio Rodríguez de Mendoza of Amazonas, were in charge of evaluating, measuring, analysing, and interpreting the perception of the attributes of sweetness, acidity, bitterness, astringency, and presence of floral and fruity notes of fresh cocoa fruits based on the criteria described in Table 3.

2.3. Relationship between the Sensory Characteristics of Fresh Fine-Flavor Native Cocoa and Altitude. To determine the relationship between the altitude of the sampling area and the organoleptic sensory characteristics of fine-aroma native cocoa, we determined three sampling elevations, low elevation: A1 (less than 500 m), medium elevation: A2 (from 501 to 800 m), and high elevation: A3 (more than 801 m), and we determined the level of presence of cocoa with floral and fruity notes according to altitudinal levels.

2.4. Statistical Analysis. The data obtained were analyzed using descriptive statistics in InfoStat software (version 2019). For qualitative data, sectorial graphs were made, while for quantitative data, frequency distribution histograms were produced. In addition, a multiple correspondence analysis allowed determining the relationship between the altitudinal levels of sampling sites and the sensory characteristics (fruity and floral notes) that differentiate fine-flavor cocoa.

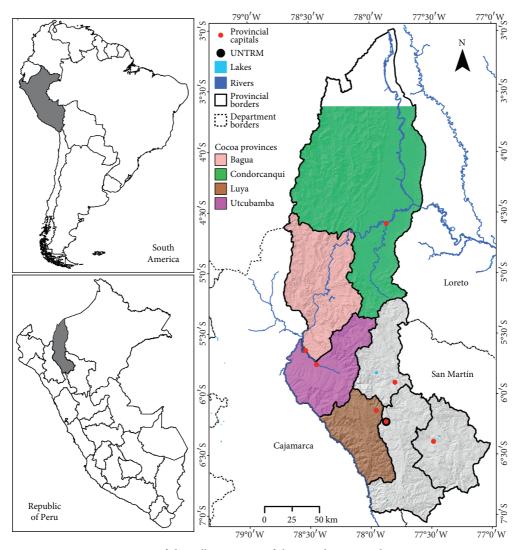


FIGURE 1: Location of the collection area of the 146 characterised FFNC ecotypes.

TABLE 1:	Morphological	descriptors of	f cocoa 1	oods and	seeds.
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Type of descriptor	Descriptor	Indicator	
Fruit characteristics	Color of unripe cocoa fruits	1: green, 2: pigmented green, 3: red, 4: pigmented red	
	Fruit shape	1: oblong, 2: elliptical, 4: spherical, 5: oblate	
	Apex form	1: attenuate, 2: acute, 3: obtuse, 5: mammelate	
	Fruit surface rugosity	0: absent, 3: slight, 5: intermediate, 7: strong	
	Basal constriction	0: absent, 3: slight, 5: intermediate, 7: strong	
	Fruit wall thickness (cm)	3: thin (<1.2), 5: intermediate (1.2–1.6), 7: thick (>1.6)	
	Ridge pair appearance	3: intermediate, 5: equidistant	
	Primary furrow depth (cm)	3: superficial (<0.5), 5: intermediate (0.5–1.0), 7: equidistant (deep) (>1.0)	
Seed characteristics	Cotyledon color	1: creamy white, 2: pinkish white, 3: violet, 4: purple, 5: white	
	Seed form in the longitudinal section	1: oblong, 2: ovate, 3: elliptical, 4: irregular	
	Seed form in the transverse section	1: flattened, 2: intermediate, 3: rounded	

3. Results

3.1. Morphological Characteristics of FFNC Fruits from Northeastern Peru. Figure 2 shows the morphological descriptions of the fruits of fine-aroma native cocoa according to color of the unripe fruit (Figure 2(a)), the shape of the fruit (Figure 2(b)), and the shape of the apex (Figure 2(c)). Regarding unripe fruit color, we found that 76.7% of the fruits were green, 17.8% were red, and 5.5% were pigmented green or red. Regarding fruit shape, 73% were elliptical, 23% were oblong, and 4% were spherical or oblate. On the contrary, 38% of the fruits had an obtuse apex, 31% were attenuated, and 30% were acute and pointed.

Descriptor	Indicator		
Fruit length	1: large (20–24 cm), 2: medium (15–19 cm), 3: small (10–14 cm).		
Seed length	1: large, 2: medium, 3: small.		
Number of seeds per fruit	We selected a sample of 20 fruits (N = 20). Subsequently, we quantified the number of seeds per fruit, and a final average was obtained.		
Dry peeled seed weight	A sample of 30 oven-dried seeds was weighed ($90^{\circ}C \times 8$ hours). Afterwards, the weight was divided by 30 to obtain the average weight of the seeds.		
Index of cocoa fruits	The formula proposed by Wood and Lass [12] was used: $IF = 1000/NUSE \times PESE$ where IF = index of cocoa fruit, NUSE = number of seeds/fruit, and PESE = average dry weight of one seed.		

TABLE 2: Productivity descriptors of fine-flavor native cocoa.

TABLE 3: Organoleptic descriptors of fine-flavor native cocoa.

Descriptor	Indicator
Sweet	1: low, 2: moderate, 3: high
Acid	1: low, 2: moderate, 3: high
Bitter	1: low, 2: moderate, 3: high
Astringent	1: low, 2: moderate, 3: high
Floral	1: low, 2: moderate, 3: high
Fruity	1: low, 2: moderate, 3: high

Regarding fruit surface roughness, 68% of fruits had light roughness, 25% had intermediate roughness, and only 5% and 1% had intense roughness and no roughness (absent), respectively (Figure 3(a)). Figure 3(b) shows the fruit wall thickness, in which 54% of fruits presented a thick peel, 24% had an intermediate peel thickness, and 22% had a thin peel. In relation to basal constriction, 49% of fruits had no basal constriction, 38% had slight basal constriction, and 13% had intermediate or intense basal constriction (Figure 3(c)).

The separation of a pair of spines and the depth of the furrows were the last variables corresponding to the characteristics of the fruits that were evaluated. Here, 97% of the ecotypes showed a pair of intermediate spines, and 3% had equidistant spines; fruits with fused and slightly separated spines were not found. Regarding the depth of the primary furrow to the ridge level (cm), 57% had an intermediate furrow depth (0.5–1.0 cm), 40% had a shallow furrow depth (<0.5 cm), and 3% had an intense furrow depth (depth > 1.0 cm) (Figure 4(b)).

3.2. Morphological Characteristics of the FFNC Seeds from the Northeastern Part of Peru. In relation to the morphological characteristics of the FFNC seeds identified, for seed shape in the longitudinal section (Figure 5(a)), 59% of the ecotypes presented an elliptical shape, 28% were oblong, and 13% were irregular or ovate. For seed shape in the transverse section (Figure 5(b)), 44% of the ecotypes presented a flattened shape, 38% had an intermediate shape, and 18% were rounded. Additionally, 71% of the ecotypes presented purple cotyledon color, 24% were violet, and 5% were creamy white, pinkish white, or white (Figure 5(c)).

3.3. Sensory Characteristics of Fresh FFNC Fruits. Sensory analysis of the 146 FFNC ecotypes showed that 68% had

moderate levels of sweetness, 30% had high levels of sweetness, and only 1% had low levels of sweetness (Figure 6(a)). Likewise, 71% of the ecotypes presented a moderate level of acidity, 22% presented a low level of acidity, and 2% presented a high level of acidity (Figure 6(b)). Regarding bitterness, 78.8% of the ecotypes were found to have low levels of acidity, 20.5% had intermediate levels of acidity, and 0.7% had high levels of acidity (Figure 6(c)).

Figure 7 shows the sensory characteristics of fresh FFNC fruits in relation to the presence of floral (Figure 7(c)), fruity (Figure 7(b)), and astringent (Figure 7(a)) notes. Here, 65% of the ecotypes had a high level of floral notes, 29% had a moderate level of floral notes, and 6% had a low level of floral notes. Similarly, 82% had a high level of fruity notes, 16% had a moderate level of fruity notes, and only 2% had a low level of fruity notes.

3.4. FFNC Productivity Characteristics. FFNC productivity characteristics in relation to fruit and seed length are shown in Figures 8(a) and 8(b), respectively, where 52% and 64% of fruits and seeds were large, 46% and 33% of fruits and seeds were medium-sized, and only 2% and 3% of fruits and seeds were small.

Regarding the productivity characteristics of FFNC ecotypes, in relation to the numerical variables, histograms were made for the number of seeds per fruit, dry seed weight, and cocoa fruit index (Figures 9(a)-9(c), respectively). The identified ecotypes tended to have 34 to 53 seeds per fruit; however, there were a few ecotypes that had 22 to 28 seeds or 53 to 66 seeds per fruit. With reference to seed weight, seeds were more likely to be 1.95 to 2.29 g, rather than 2.64 g to 2.98 g. Therefore, characteristics such as seed length and weight allowed us to find the cocoa cob index. It can be observed that approximately 30% of the ecotypes had a cocoa fruits would be required to obtain one kilogram of fermented dry bean; fewer proportion of the ecotypes had a cob index of 24 to 26.

3.5. Relationship between the Sensory Characteristics of Fine-Flavor Cocoa and Altitude. The first two axes of the multiple correspondence analysis (MCA) explained 41.79% of the variability of the sample (Figure 10). This first axis separates the A3 ecotypes, those that have moderate floral notes, from

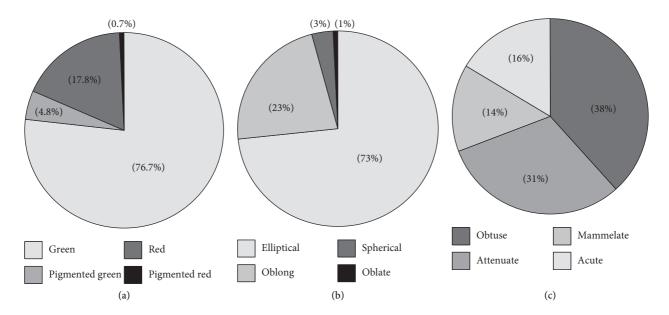


FIGURE 2: Morphological characteristics of the fruit of 146 fine-flavor native cocoa ecotypes. (a) Color of immature fruit. (b) Fruit shape. (c) Apex shape.

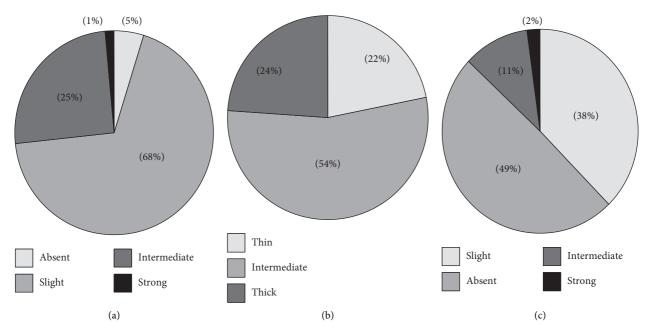


FIGURE 3: Morphological characteristics of the fruit of 146 ecotypes of fine-flavor native cocoa. (a) Fruit surface rugosity. (b) Fruit wall thickness. (c) Basal constriction.

the A2 ecotypes, those that have high floral and fruity notes. The A1 ecotypes, on the contrary, do not have a clear association with any of the sensory notes evaluated.

Therefore, there is a positive relationship between altitude and the presence of floral and fruity notes; in other words, the higher the altitude of the sampling areas, the higher the presence of floral and fruity notes, typical characteristics of fine-flavoured cocoa.

4. Discussion

In the present study, the variability of morphological traits such as fruit color, shape, roughness, and length allows for the existence of a great diversity of ecotypes in northeastern Peru. Thus, morphological descriptors applied during cocoa characterization facilitate the identification, differentiation, and selection of germplasm diversity [13, 14], highlighting the particular characteristics expressed by each genotype [15, 16]. Based on the results, the best fruit characteristics were an elliptical shape (73%), green immature fruit color (76.7%), a slight roughness (68%), and a ridge pair appearance (97%). These characteristics are similar to those of the Criollo ecotypes of the Cajamarca region [17] and the cultivars of the Peruvian cocoa catalog [18]. However, morphological characterization cannot be taken as a definitive identification but must be part of the germplasm

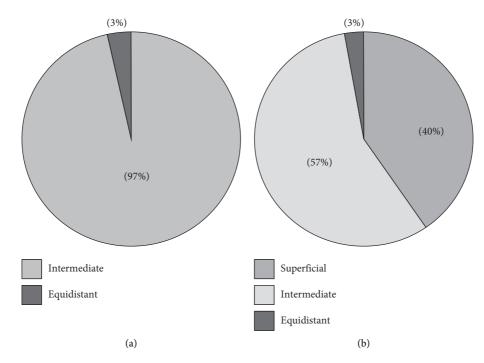


FIGURE 4: Morphological characteristics of the fruit of 146 FFNC ecotypes. (a) Ridge pair appearance. (b) Primary furrow depth.

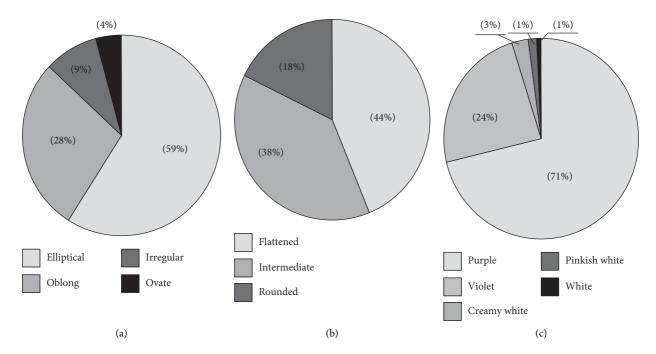


FIGURE 5: Morphological characteristics of the seeds of 146 fine-flavor native ecotypes. (a) Seed form in the longitudinal section. (b) Seed form in the transverse section. (c) Cotyledon color.

classification process [19] since this process requires a high level of expertise from the evaluator, considering that the descriptors are subjective and can result in erroneous comparisons or ratings [19, 20].

On the contrary, Pavon [21] mentioned that the characteristics with the greatest discriminating power are those inherent to the fruit (color, shape, length, and wall thickness). According to García and Fernando [18], the elliptical and oblong shape is the most common characteristic in a cocoa collection, which makes it difficult to differentiate between germplasm groups, since the elliptical shape is shown by the accessions "Alto Amazonas" and "Trinitario." However, the shape of the fruit, along with characteristics such as basal constriction and low roughness,

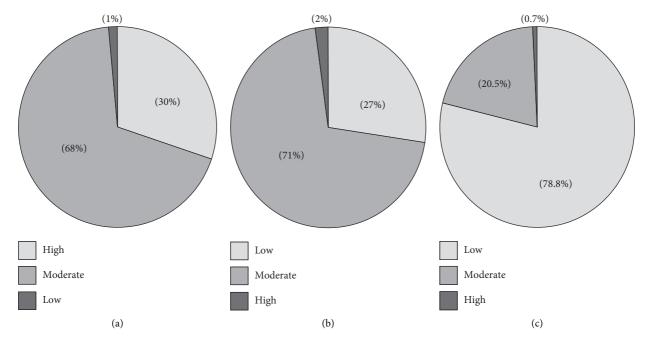


FIGURE 6: Sensorial characteristics of fresh fruits from 146 FFNC ecotypes. (a) Sweet. (b) Acid. (c) Bitter.

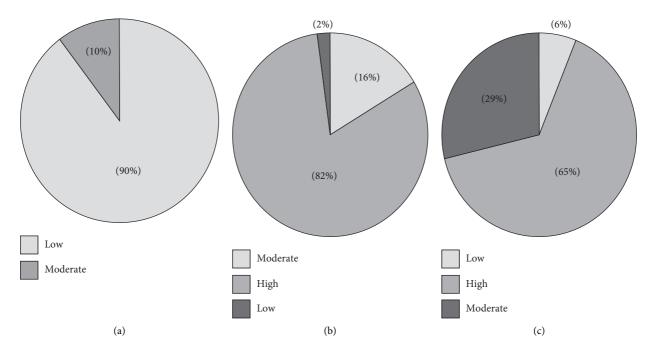


FIGURE 7: Sensorial characteristics of fresh fruits from 146 FFNC ecotypes. (a) Astringent. (b) Fruity. (c) Floral.

reflects the hybridization of native × Forastero cacao from the "lower Amazon" that has developed in these plantations over time [22].

Regarding seed color, purple (71%) and violet (24%) cotyledons were the most prominent characteristics; these characteristics are similar to those reported in promiscuous ecotypes located in the provinces of Jaén and San Ignacio, Cajamarca, Peru [17]. Purple color is generally associated with the group of Forastero and Trinitario cocoas. These variations may be related to the influence exerted by the Forastero and Trinitario cocoas on the characteristics of Criollo cocoa. In fact, white cocoa has a low prevalence (1%) in northeastern Peru; however, this result confirms what was mentioned by Doster et al. [23], suggesting that, in the north of the country, there is a variety known as "porcelain" whose main characteristic is white color of the cotyledon (white cocoa). According to a report by MINCETUR [24], in the Amazon region (Bagua and Utcubamba), 20% of cocoa plantations belong to breeders who use plants with different genetic origins. According to Quiñones et al. [25], cacao beans have important characteristics that can be used in the

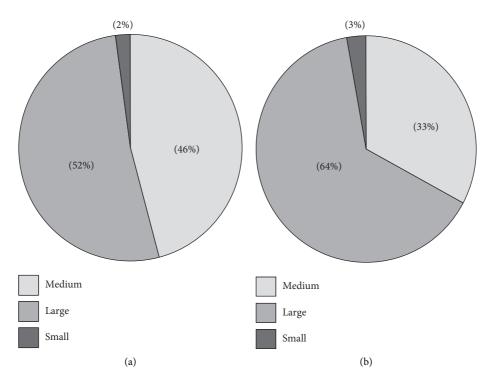


FIGURE 8: Productivity characteristics of 146 FFNC ecotypes. (a) Fruit length. (b) Seed length.

discrimination of genotypes or cultivars. Likewise, the introduction of cocoa cultivars plays an important role in crosspollination due to the self-incompatibility present in some trees [26] and in the diversification of the cocoa population by crossbreeding of plant material [27]. Consequently, the traditional method of propagation (seeds) ensures a high degree of variability due to the combination of genes from both parents [15, 28]. One factor that differentiates Criollo-type cacao fruits is their lower number of total seeds and thinner wall thickness as Forastero and Trinitario cacaos are not distinguished by these characteristics [15].

In terms of sensory attributes, fine-flavor Criollo-type cocoa fruits stand out for their high levels of floral (65%) and fruity (82%) notes, moderate levels of sweetness and acidity (71%), and low level of astringency (90%) [29]. Flavor and aroma are inherent characteristics of each cocoa genotype; however, these attributes may undergo variations due to plant-environment interactions [30, 31]. In addition, temperature is an important factor because it affects the formation and content of aroma and flavor precursors throughout fruit development [32, 33]. Likewise, factors such as fermentation, drying, and processing [34-36] also play an important role in the quality of cocoa products. Desirable sensory characteristics of cocoa beans, nuts, fresh fruits, and dry seeds are directly related to proper harvest and postharvest processes, especially during fermentation, and the genotype used [37]. Thus, the difference between fine-flavor cocoa and bulk or ordinary cocoa, effectively, lies in the flavor, rather than in the other quality aspects, with rich and balanced chocolate bases [38]. Much fine-flavor

cocoa comes from varieties of Criollo and Trinitario cocoa trees; therefore, special flavours are essential in the production of top-quality chocolates [39].

Taking into account the agronomic descriptors, we found that more than 50% of the fruits contained between 35 and 53 seeds; similar values were reported for cocoa clones from the INTA Technology Development Center in Nicaragua [40]. Likewise, it can be affirmed that the cacao accessions in this study possess a high productive potential since the index of cacao fruits is in the range of 14 to 16; these values are considered within acceptable levels for the productive characteristics of cacao [41, 42]. Large fruits (52%) and seeds (64%) are also attributes that provide good productivity potential. According to Nauca and Jose [43], in the morphological characterization of cocoa ecotypes, there is slight to moderate phenotypic variation with respect to fruit shape, basal constriction, apex shape, fruit surface roughness, fruit wall thickness, and anthocyanins on the back of the fruits; within the quantitative characteristics, the cocoa fruit index, potential yield, and actual yield can vary between clones. Likewise, pod production is higher in the rainy season [44]. Total pods, yield, and indirect cocoa yield efficiency are correlated with [45]. Therefore, it can be stated that the use of descriptors for cocoa is necessary for the following reasons: (1) to standardize descriptive terminology to allow the exchange of information among researchers, (2) to make a simple inventory available to all researchers, (3) to help the breeder select the best accessions for the breeding program, and (4) to simplify collection management and maintenance [46].

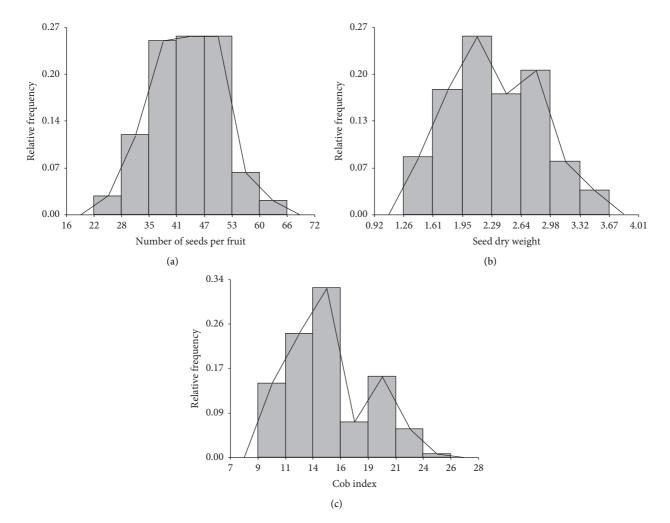


FIGURE 9: Histograms of the productivity characteristics of FFNC ecotypes. (a) Number of seeds per fruit. (b) Dry peeled seed weight. (c) Index of cocoa fruits.

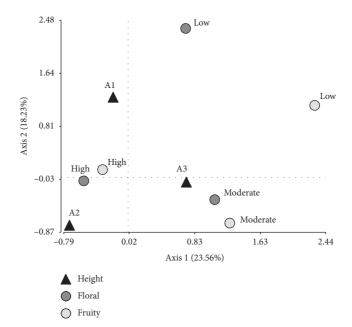


FIGURE 10: Multiple correspondence analysis for sampling altitude (A1: less than 500 m.a.s.l; A2: from 501 to 800 m.a.s.l; A3: above 800 m.a.s.l.), floral notes (low, moderate, and high), and fruity notes (low, moderate, and high).

The optimal altitudinal range for the production of fineflavor cocoa is between 500 and 900 metres above sea level; nonetheless, fine-flavor cocoa can also be produced as low as sea level and as high as 1200 metres [47]. Peru, being located close to the equator, has the agroclimatic conditions to grow cocoa at altitudes of up to 1400 metres above sea level [48]. In this sense, the positive relationship between altitude and the presence of fine-flavoured Criollo cocoa (with floral and fruity notes) above 501 metres above sea level explains that altitude is a determining variable for the quality of cocoa beans. At higher altitudes, cold temperatures are more frequent; therefore, the growth cycle of the plant is slower, which extends the development of the beans, making them synthesise more complex sugars and producing more attractive and deeper flavours [49]. In coffee, however, not only does altitude contribute positively to the increase in chemical composition, especially sucrose content [50], but soil characteristics such as phosphorus (P) and cation exchange capacity (CEC) also influence the sensory quality of coffee [51]; this means that coffee grown at higher altitudes has a higher market value [52]. This, consequently, represents an alternative for the production of fine, aromatic Criollo cocoa at high altitudes that meets the needs of special markets.

5. Conclusions

The morphological characteristics of the fruits that stand out are the presence of green color of unripe fruit (76.7%), elliptical fruit shape (73%), slight fruit surface rugosity (68%), intermediate fruit wall thickness (54%), intermediate basal constriction of the fruit (49%), ridge pair appearance (90%), and intermediate primary furrow depth (57%). For the morphological characteristics of the seeds, we can point out that 59% have an elliptical seed form in the longitudinal section, 82% have a flattened and intermediate seed form in the transversal section, and 71% of them display purple color of the cotyledon. On the sensory characteristics, the ecotypes identified are characterised by a moderate level of sweetness and acidity, a low level of bitterness and astringency, and high levels of floral and fruity notes. Regarding the productivity characteristics, both fruits and seeds of FFNC ecotypes have a significant length and an excellent index of cocoa fruits (14-16), resulting in a positive productivity level. Medium-altitude and high-altitude areas, ranging from 501 m.a.s.l. onwards, constitute the agroecosystems that host the largest number of FFNC ecotypes, characterised by the moderate and high presence of floral and fruity notes.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

M. O. and M. Q. conceptualized the study and contributed to funding acquisition. M. O., L. G., and P. E. developed the methodology. M. G. and M. O. performed formal analysis and wrote the original draft. C. A., R. R., and P. E. contributed to research. M. Q., R. R., C. A., L. G., and M. G. reviewed and edited the article. All authors read and accepted the published version of the manuscript.

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